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ORIGINAL ARTICLE

Esculetin Ameliorates Cognitive Impairments in D-Galactose-Induced Alzheimer's Disease Rats by Inhibiting Inflammation and Oxidative Stress

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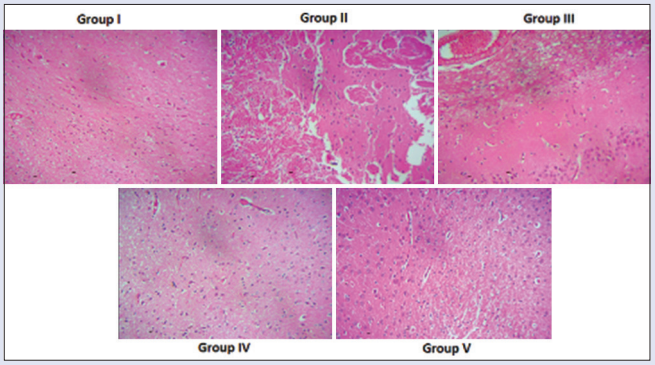
ABSTRACT

Background: Alzheimer disease (AD) is a common form of dementia and is described by memory loss and behavioral disorder. The prevalence of AD is increasing rapidly each year worldwide. **Objectives:** In this study, we aimed to discover the therapeutic properties of esculetin against D-galactose (D-gal)-induced AD in an animal model. **Materials and Methods:** AD was initiated in rats by administering 150 mg/kg of D-gal via subcutaneous route for 6 weeks and supplemented with 10, 20, and 30 mg/kg of esculetin, respectively. Subsequently, memory and learning of the rats were investigated using the Morris water maze (MWM). The organ index of the liver, spleen, thymus, and kidneys was assessed. The enzyme activities of superoxide dismutase (SOD), catalase (CAT), GSH-Px, and heme oxygenase-1 (HO-1) and the levels of advanced glycation end products (AGEs), 8-iso-prostaglandin F (8-iso-PGF), and 8-hydroxy-2-deoxyguanosine (8-OHdG) were assessed using commercially available kits. The level of acetylcholine (Ach) and the activity of acetylcholinesterase (AChE) was also assessed using kits. The brain tissue samples were assessed microscopically. **Results:** According to the results, esculetin significantly improved the bodyweight and organ index in AD animals. It significantly modulated the spatial learning and memory and improved the activities of CAT, SOD, GSH-Px, and HO-1. It significantly reduced the contents of AGEs, 8-iso-PGF, and 8-OHdG and inflammatory markers. Furthermore, esculetin increased the level of ACh and the reduced activity of AChE. Histological analysis of the brain tissue revealed that esculetin attenuated the D-gal-induced histological changes in the brain of AD rats. **Conclusion:** The findings of this study reveal that esculetin can ameliorate inflammation and oxidative damage in D-gal-induced AD rats. It can be further explored as a therapeutic agent to treat AD.

Key words: 8-iso-PGF, 8-OHdG, esculetin, neuroinflammation, neurotransmitters, spatial memory

SUMMARY

- Alzheimer's disease is the most common form of age-associated dementia and is described by behavioral disorder and memory loss.
- Esculetin can attenuate oxidative damage and neuroinflammation by elevating neuronal antioxidant enzyme activities, decreasing oxidative damage biomarkers, reducing pro-inflammatory cytokines' levels, and triggering cholinergic mechanisms in D-gal provoked AD animals.



Abbreviations used: AD: Alzheimer's disease; D-gal: D-galactose; MWM: Morris Water Maze; ChAT: Choline acetyltransferase; Ach: Acetylcholine.

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INTRODUCTION

Alzheimer disease (AD) is the most common form of age-associated dementia and causes behavioral disorder and memory loss. Worldwide, the estimated prevalence of dementia was approximately 46.8 million in 2015, which is predicted to double every 20 years, that is, approximately 74.7 million people in 2030 and 131.5 million in 2050 might suffer from AD.[1] The pathological changes in patients with AD primarily comprise neurotransmitter disorders, nerve fiber tangles and plaques, inflammatory responses, and severe oxidation.[2] In addition, aging is the primary cause of AD. Furthermore, oxidative stress is the most crucial factor in the development of AD due to the increased accumulation of reactive oxygen species (ROS).[3,4] The level of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the brain tissues is comparatively low, which makes it highly prone to ROS-mediated oxidative damage.[5] Furthermore, a previous study reported that astrocytes and microglia release high quantities of pro-inflammatory biomarkers such as interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α.[6] IL-1β is the most important inflammatory mediator during neuroinflammation and is believed to be the root cause of dementia. The level of IL-1β in the cerebrospinal fluid and serum of patients with AD has been found to be increased.[7] IL-1β can stimulate

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the astrocytes and microglia, which in turn triggers increased production of inflammatory mediators, which leads to neurotoxicity. These reports suggest that oxidative stress and inflammation play an important role in the development of AD.[8,9]

According to the literature, the cholinergic system is responsible for various cognitive mechanisms, in which the acetylcholine (ACh), a neurotransmitter, is generated by the choline acetyltransferase (ChAT). Subsequent to the depletion of cholinergic neurons, the discharge of ACh reduces and results in learning difficulties. During the progression of AD, the prefrontal cortex and hippocampal cholinergic neurons are injured, which leads to the reduced generation and discharge of AC. This results in cognitive impairments and memory loss.[10]

Previous studies have shown that chronic exposure to D-galactose (D-gal) (mild dosage) can cause changes that resemble the natural process of aging in animal models, such as cognitive defect, oxidative stress, changes in gene expressions, and reduced immune responses.[11–14] It increases the process of neurodegeneration by increasing the activity of acetylcholinesterase (AChE), increasing the level of ROS, increasing cognitive impairment, and decreasing the activity of antioxidant enzymes.[15] Neurotoxicity developed due to the prolonged exposure to D-gal in animals has been widely employed as an experimental model for the investigation of mechanisms of development of AD.[16]

Esculetin is a coumarin derivative, which occurs in numerous herbal plants such as *Citrus limonia*, *Fraxinus rhynchophylla*, and *Fraxinus chinensis*.[17] Esculetin exhibits various activities such as antioxidant, anti-inflammatory, and anti-fibrotic,[18] anti-tussive,[19] anti-anxiety, anticancer,[20] antinociceptive,[21] antidiabetic, antimicrobial, and anticoagulant.[22] It is also effective against non-communicable diseases,[23] rheumatoid and osteoarthritis,[24] psoriasis,[25] and sepsis.[26] However, so far, there are no studies that discuss the therapeutic roles of esculetin against AD. Therefore, in this study, we aimed to discover the beneficial properties of esculetin against the D-gal-induced AD model in rats via inhibition of oxidative stress and inflammation.

MATERIALS AND METHODS

Chemicals

Esculetin (percentage purity: 98%), D-gal, buffered saline, and additional chemicals were purchased from Sigma-Aldrich, USA. Marker-specific assay kits were purchased from Thermo Fisher Scientific and MyBioSource (USA).

Experimental rats

In this study, healthy male Sprague–Dawley rats (10–12 weeks old) weighing around 160–210 g were obtained from the institutional animal house. The animals were maintained in well-sanitized infection-free polypropylene cabins and were maintained under laboratory conditions (22°C–24°C and 12-h light/dark cycles). Throughout the experimental period, all rats were given free access to food and water. The protocols were approved by the institutional animal ethical committee (approval number: 20-1071). Before conducting the experiments, all rats were acclimatized for a week under laboratory conditions.

Experimental groups

The rats were randomly divided into five groups containing six rats each: control (group I), AD model (group II), and AD+esculetin-treated groups (groups III–V). Group I animals were excluded from all the treatments and administered only with saline. Group II animals were subcutaneously administered with D-gal at 150 mg/kg bodyweight once a day for 6 consecutive weeks to induced AD.[27] Group III–V animals were supplemented with 10, 20, and 30 mg/kg of esculetin, respectively, by oral route for 6 weeks after the administration of D-gal. The animals from the control and AD groups were supplemented with the same quantity of saline solution without the esculetin. The bodyweight of each animal was carefully weighed before and after the experiments and data were tabulated.

Morris Water Maze (MWM) test

The memory and spatial learning of the experimental rats were assessed by the technique of MWM. The maze was designed with a height of 60 cm diameter of 150 cm and separated into four equivalent quadrants and filled with water at 40-cm depth. By using non-toxic water-soluble black ink, the water was made opaque. The portable escape platform was submerged in water at the center of the quadrant. The pool was located in a low-light-powered cabin with attached distal visual signs, which provided a route-finding key to locate the target. During the acquirement time, a 2-min training trial was given to the rats twice/day for 4 consecutive days. The animals were permitted freely to reach the escape platform within 2 min for each training period. The animals were permitted to stay on the platform for 10 s once they found it. If rats failed to reach the platform, they were directed to reach the same and stay on for 30 s. The time periods required by the rats to identify the platform (escape latency) were utilized as the extent of spatial learning. After 24 h of the last training, the probe test was executed, where all animals were permitted to examine the pool for 2 min in the absence of the platform. The time expended by a rat in the platform was recorded and used to assess the reference memory.[28]

Organ index measurement

All the animals were sacrificed under ketamine/xylazine (90/10 mg/kg) anesthesia after the physical experiments and then internal organs such as the liver, spleen, thymus, and kidneys were dissected out from both control and experimental rats. Then, the excised organs were weighed accurately to detect the organ index, and the final data are represented as mg/g.

Measurement of antioxidant enzyme activity

The SOD, CAT, GSH-Px, and HO-1 activities in both control and experimental rats were examined with the aid of marker-specific assay kits as per the guidelines described by the manufacturer (Thermo Fisher Scientific, USA).

Measurement of oxidative biomarkers

The contents of AGEs, 8-iso-PGF, and 8-OHdG in the serum of control and experimental rats were assessed with the help of marker-specific assay kits (Abcam, UK).

Quantification of inflammatory markers

The contents of IL-1β, IL-6, and TNF-α in the brain tissues of experimental rats were examined by using the commercial kits (MyBioSource, USA).

Assay of ACh and AChE in the brain tissue

The content of ACh and activity of AChE in the brain tissues of experimental rats were assessed using assay kits. For this, the excised brain tissues from both control and treated animals were homogenized with 0.25 M of sucrose buffer for 30 min. Then this suspension was centrifuged at 10,000 rpm and the supernatant was used to assess the ACh level and AChE activity by using the spectrophotometric technique and absorbance was taken at 412 nm.

Histopathological study

The hippocampal tissues were excised from the experimental rats and cut into small pieces. Then, the tissues samples were fixed in the Bouin's fixative for 24 h at 37°C. Subsequently, the samples were paraffinized and cut into 4–6-µm-thick sections, stained with hematoxylin and eosin, and observed under a microscope (40×).[29]

Statistical analysis

The data were analyzed using GraphPad Prism (GraphPad Software, Inc., San Diego, USA) version 9.0 and presented as mean ± standard deviation (SD) of triplicates. The differences between treatment groups were assessed by one-way analysis of variance (ANOVA) and Tukey's *post hoc* assay. Significance was fixed at $P < 0.05$.

RESULTS

Effect of esculetin on the bodyweight and organ index in the experimental animals

Figure 1 shows the effect of esculetin on the bodyweight and organ index in the D-gal-induced AD animals. According to the results, there was a notable reduction in the bodyweight and organ index of the spleen, thymus, liver, and kidneys when compared with the control animals. Interestingly, these reductions were effectively ($P < 0.05$) ameliorated by esculetin. The supplementation of 10, 20, and 30 mg/kg of esculetin appreciably ($P < 0.05$) enhanced the bodyweight and organ indexes of the spleen, thymus, liver, and kidneys weights in the D-gal-induced AD animals [Figure 1].

Effect of esculetin on the memory and spatial learning capacities in the experimental rats

Figure 2 depicts the memory and learning capacities of the control and experimental rats as assessed by the MWM test. The D-gal-induced AD rats have notable augmentation in the escape latency, diminution in the time spent in the target quadrant, and crossing frequency when compared with the control animals. Esculetin (10, 20, and 30 mg/kg) significantly reduced the reduction in the escape latency and improved the time spent by the experimental rats ($P < 0.05$). These results demonstrate the beneficial properties of esculetin in restoring memory and cognition in the D-gal-induced AD animals [Figure 2].

Effect of esculetin on the enzymatic antioxidants in the experimental rats

Figure 3 depicts the effects of esculetin on the activities of enzymatic antioxidants in the D-gal-induced AD animals. The D-gal-induced AD animals exhibited a drastic reduction in the CAT, SOD, HO-1, and GSH-Px activities in the brain tissues when compared with the control animals. Esculetin (10, 20, and 30 mg/kg) significantly restored the activities of CAT, SOD, HO-1, and GSH-Px activities in the brain tissues of D-gal-induced AD animals ($P < 0.05$) [Figure 3]. These results provide evidence of the antioxidant property of esculetin against the D-gal-induced AD in rats.

Effect of esculetin on the oxidative biomarker levels in the experimental rats

Figure 4 shows the effect of esculetin treatment on the contents of oxidative biomarkers such as AGEs, OhdG, and 8-iso-PGF in the serum of D-gal-induced AD animals. There was a drastic increase in the level of AGEs, OhdG, and 8-iso-PGF in the serum of D-gal-induced AD animals when compared with the control animals. Esculetin (10, 20, and 30 mg/kg) significantly attenuated the level of AGEs, OhdG, and 8-iso-PGF in D-gal-induced AD animals ($P < 0.05$) [Figure 4]. This result demonstrates the antioxidant activity of esculetin.

Effect of esculetin on the level of the pro-inflammatory markers in the experimental animals

Figure 5 shows the level of IL-6, IL-1β, and TNF-α in the brain tissues of experimental rats. The D-gal-induced AD animals have a drastic augmentation in the contents of IL-6, IL-1β, and TNF-α when

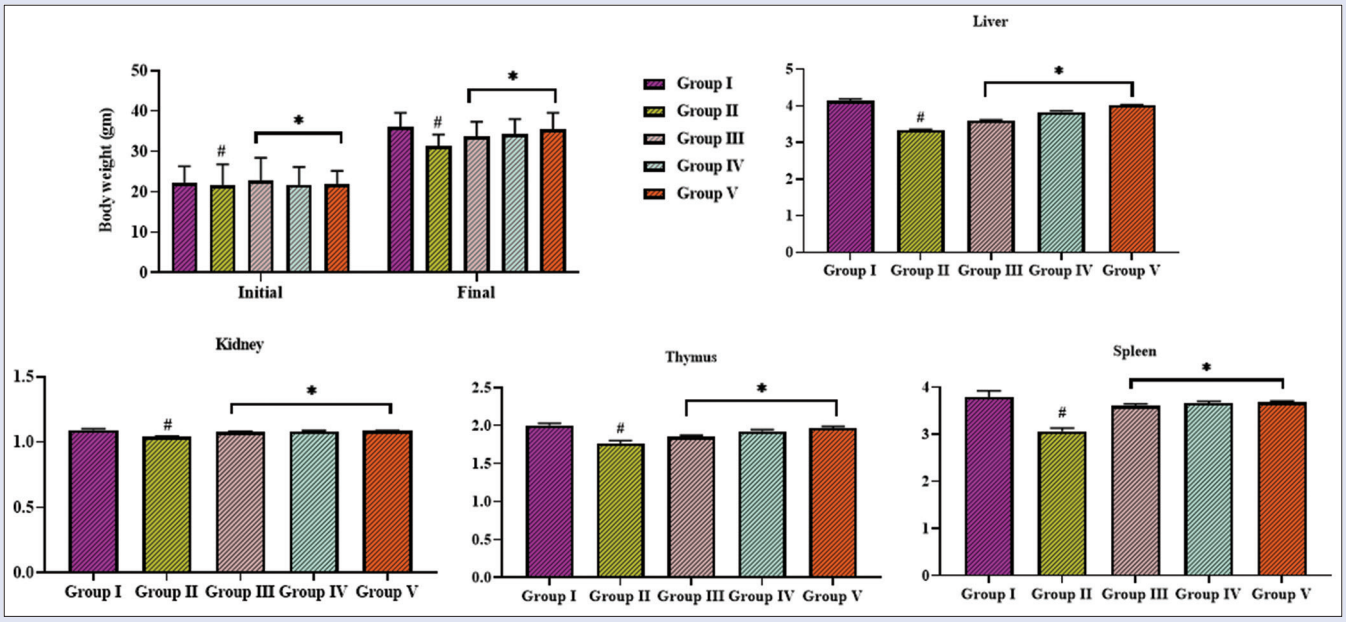

Figure 1: Effect of esculetin on the bodyweight and organ index in the experimental rats. Data are displayed as the mean ± SD of triplicate measurements and statistically scrutinized by one-way ANOVA and Tukey's *post hoc* assay. "#" represents that data varied significantly at $P < 0.05$ from control, and "*" represents that data varied significantly at $P < 0.01$ from the AD group

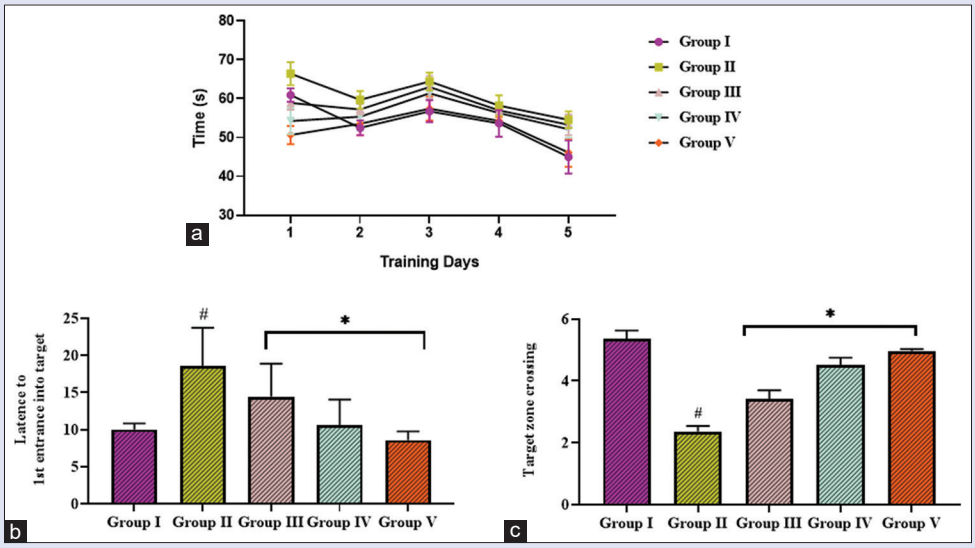

Figure 2: Effect of esculetin on the memory and spatial learning capacities in the experimental rats. Data are displayed as the mean ± SD of triplicate measurements and statistically scrutinized by one-way ANOVA and Tukey's *post hoc* assay. "#" represents that data varied significantly at $P < 0.05$ from control, and "*" represents that data varied significantly at $P < 0.01$ from the AD group

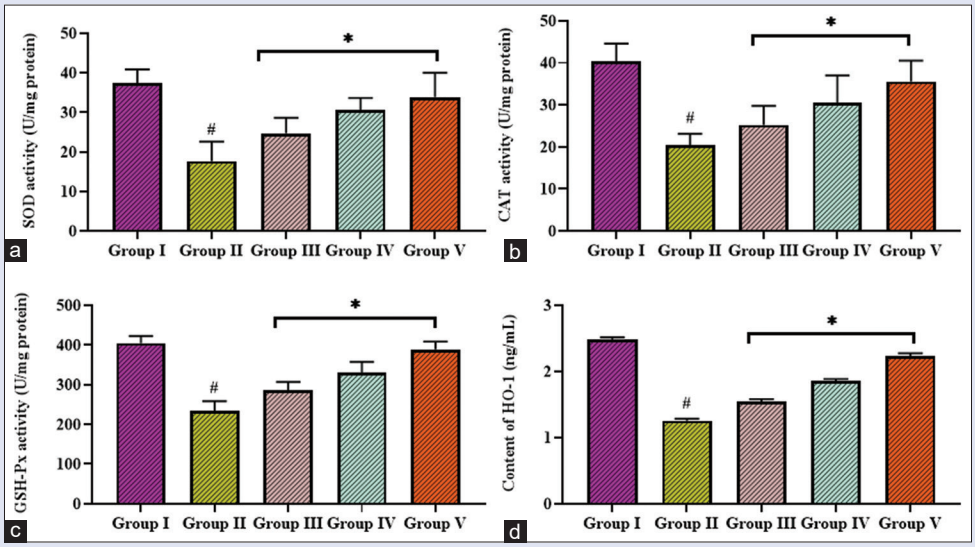

Figure 3: Effect of esculetin on the enzymatic antioxidants in the brain tissues of experimental rats. Data are displayed as the mean ± SD of triplicate measurements and statistically scrutinized by one-way ANOVA and Tukey's *post hoc* assay. "#" represents that data varied significantly at $P < 0.05$ from control, and "*" represents that data varied significantly at $P < 0.01$ from the AD group

related with the control. Esculetin (10, 20, and 30 mg/kg) significantly reduced the contents of IL-6, IL-1β, and TNF-α in the brain tissues of D-gal-induced AD animals ($P < 0.05$) [Figure 5]. These results provide evidence of the anti-inflammatory activity of esculetin.

Effect of esculetin on the ACh content and AChE activity in the experimental animals

Figure 6 shows the effect of esculetin on the ACh content and AChE activity in the D-gal-induced AD animals. According to the results, the level of ACh decreased and the activity of AChE increased in the D-gal-induced AD animals. Esculetin (10, 20, and 30 mg/kg) significantly increased the level of ACh and reduced the activity of AChE in D-gal-induced AD animals ($P < 0.05$) [Figure 6]. These results demonstrate the beneficial effects of esculetin against the D-gal-induced neurotoxicity in rats.

Effect of esculetin on the brain histopathology of experimental animals

Figure 7 shows the effects of esculetin on the histopathology of brain tissues. The control animals demonstrated the normal hippocampal neurons with a tight arrangement and intact morphologies. The pyramidal neurons exhibited large and round nuclei. However, in D-gal-induced AD animals, there was severe injury to the hippocampal region. The AD animals also demonstrated increased intercellular gaps, loosely arranged cells, shrunken pyramidal neurons with minimal cytoplasm. Interestingly, these histological changes were effectively reduced by

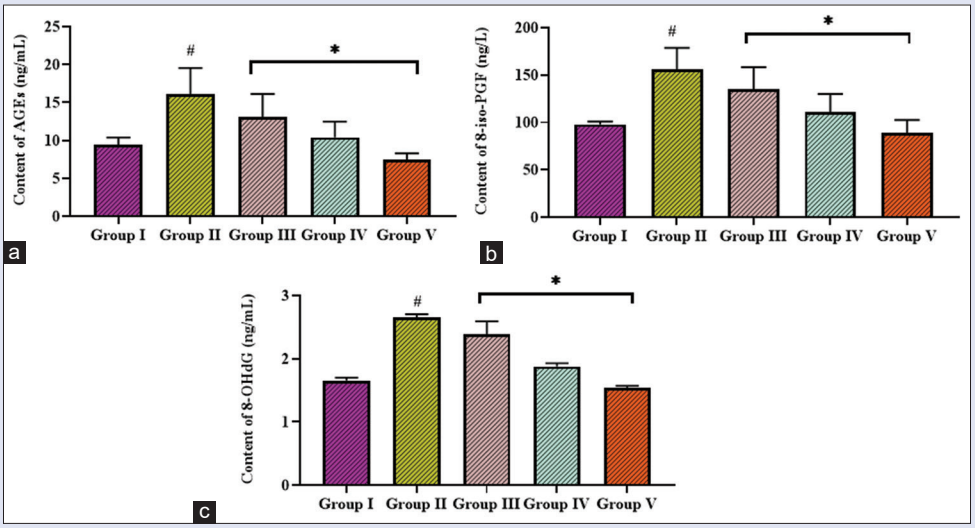

Figure 4: Effect of esculetin on the oxidative biomarker levels in the experimental rats. Data are displayed as the mean ± SD of triplicate measurements and statistically scrutinized by one-way ANOVA and Tukey's *post hoc* assay. "#" represents that data varied significantly at $P < 0.05$ from control, and "*" represents that data varied significantly at $P < 0.01$ from the AD group

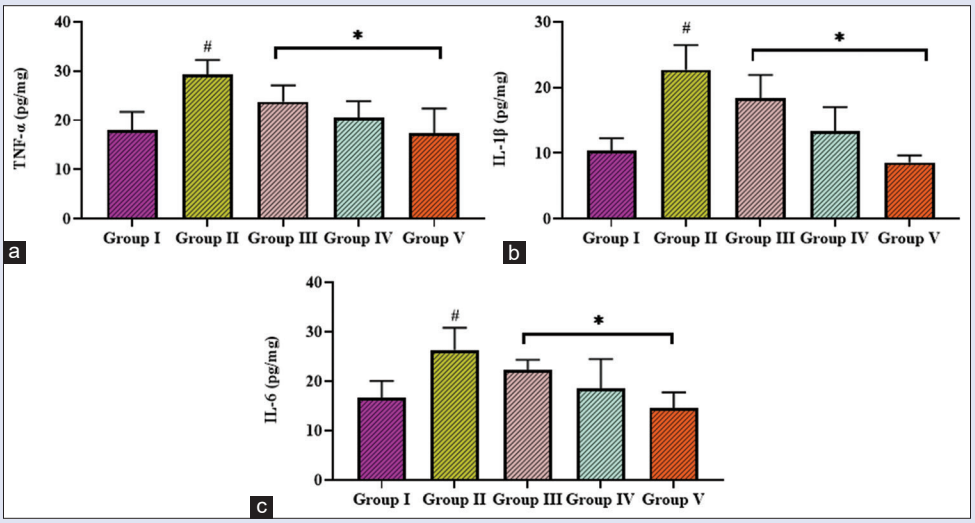

Figure 5: Effect of esculetin on the level of pro-inflammatory cytokines in the experimental rats. Data are displayed as the mean ± SD of triplicate measurements and statistically scrutinized by one-way ANOVA and Tukey's *post hoc* assay. "#" represents that data varied significantly at $P < 0.05$ from control, and "*" represents that data varied significantly at $P < 0.01$ from the AD group

esculetin (10, 20, and 30 mg/kg) [Figure 7]. These results provide evidence to the beneficial effects of esculetin against D-gal-induced neurotoxicity.

DISCUSSION

AD is characterized by the slow development of cognitive impairment and mood disorder with difficulties in daily life tasks and reduced social connections.[30] The principal cause of AD is not yet understood clearly, but there are several factors such as inflammation, oxidative stress, and cholinergic dysfunction that are responsible for the initiation and progression of AD.[31] A previous study has reported that the over-accumulation of D-gal can result in neurotoxicity, stimulation of astrocytes, neuronal apoptosis, and oxidative stress.[32] The continued exposure of D-gal in animals can speed up the process of aging and worsen cognition and motor activities, which is more similar to the signs of natural aging.[33] A continuous administration of D-gal triggers aging of the brain and speeds up the process of aging.[34,35]

In the case of the normal aging process, the brain normally undergoes structural and functional changes, which affect the synaptic and dendritic networks, blood flow, and metabolism of several neurotransmitters that lead to weakened neurotransmission. These changes can be observed in the case of behavioral changes such as memory, learning, sleep, sensory, and motor activities. Furthermore, cholinergic systems are drastically affected during the aging process.[36,37] Behavioral changes are the most sensitive factors for the assessment of memory and cognitive impairments.[38] Our results show that the D-gal-induced AD animals exhibited a drastic elevation in the escape latency and diminished time spent and crossing frequency. Interestingly, esculetin significantly decreased the escape latency and

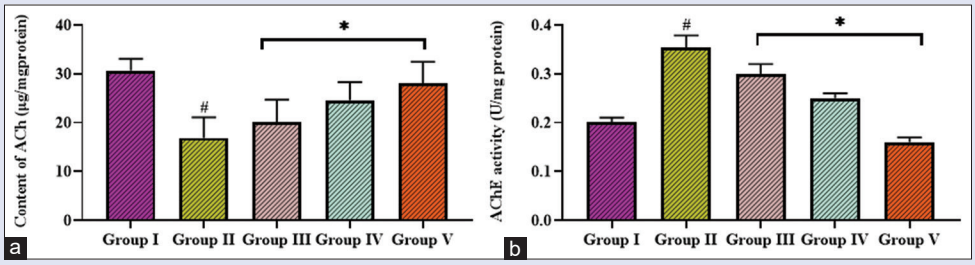

Figure 6: Effect of esculetin on the ACh content and AChE activity in the brain tissues of experimental rats. Data are displayed as the mean ± SD of triplicate measurements and statistically scrutinized by one-way ANOVA and Tukey's *post hoc* assay. "#" represents that data varied significantly at $P < 0.05$ from control, and "*" represents that data varied significantly at $P < 0.01$ from the AD group

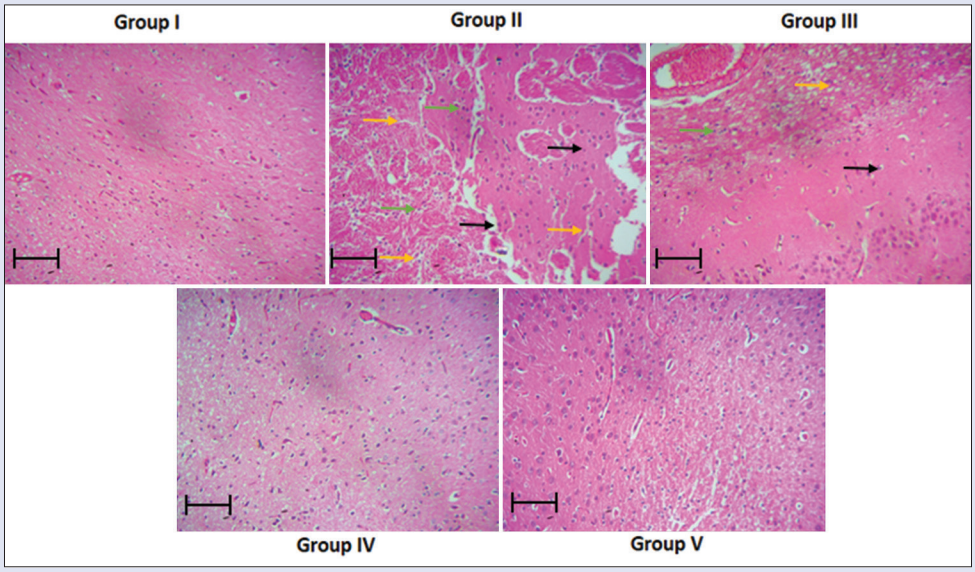

Figure 7: Effect of esculetin on the brain histopathology of experimental rats. Group I: Control rats exhibited normal hippocampal neurons with intact morphologies. Group II: Severe injury, increased intercellular gaps (yellow arrows), loosely arranged cells (green arrows), shrunken pyramidal neurons with minimal cytoplasm (black arrows) were noted in the brain tissues of D-gal-provoked AD rats. Groups III–V: Treatment with 10, 20, and 30 mg/kg of esculetin effectively rescued the D-gal-provoked alterations in the brain tissues of AD rats. *Scale bar = 50 µm, Magnification = 40×

time spent by the experimental animals [Figure 2]. This proves the neuroprotective effect of esculetin in regaining memory and lessening cognitive impairment. These results agree with a previous study.[39]

Free radicals are normally produced by cells under regular physiological circumstances. These free radicals are continuously eliminated by various intracellular antioxidants to maintain cellular homeostasis.[40] An over-accumulation of free radicals and depleted antioxidant mechanisms may result in damaging effects on several organ systems, including the brain. In the brain, they can trigger the neurodegenerative mechanisms and speed up the aging process.[41] An increased level of oxidative stress enhances neurotoxicity through several pathological mechanisms; therefore, oxidative stress has been extensively highlighted to be a critical factor in the pathophysiology of several neurological diseases.[42–44] Literature provides evidence that oxidative stress is the major cause of AD[45] and that increased oxidative stress triggers the process of neurodegeneration and neuroinflammation in age-associated ailments such as AD.[46]

The antioxidant defense mechanisms in cells are primarily regulated by Nrf2, which stimulates the transcription of genes responsible for cytotoxicity and oxidative stress.[47,48] The brain employs several enzymatic and non-enzymatic antioxidants and free radical quenching systems to guard against oxidative stress. CAT, SOD, and GSH-Px are the most important enzymatic antioxidants that actively decrease the ROS contents and protect against oxidative stress.[49,50] A previous report highlighted that D-gal triggers aging in several animal models via activation of neuroinflammation and oxidative stress that exaggerate the aging process.[51] Similarly, our results also demonstrated that the D-gal-induced AD rats exhibited a drastic reduction in the CAT, SOD, HO-1, and GSH-Px activities. Interestingly, the supplementation of 10, 20, and 30 mg/kg of esculetin significantly improved the CAT, SOD, HO-1, and GSH-Px activities [Figure 3]. These results prove the antioxidant potential of esculetin.

Cholinergic neurons are present in the basal and medial septal nucleus of the brain, and they transport large quantities of ACh to the hippocampus and cerebral cortex via projecting fibers. These neurons play an important role in memory and learning.[52,53] ACh is the most crucial neurotransmitter associated with learning, memory, and cognition, whereas AChE is accountable for ACh degradation, and its function is normally improved during AD development.[54] The depletion of ACh is supposed to be a direct cause of memory and cognitive impairments in AD. Hence, AChE inhibitors receive greater attention in AD treatment to reinstate the ACh level in the brain and recover memory and cognition difficulties of patients with AD. D-gal administration can increase the activity of AChE and decrease the level

of ACh in the brain tissues.[55,56] In this study, we observed substantial learning and memory impairments in the D-gal-induced animals. This suggests that the enhancement of learning and memory capabilities may be partially connected with the inhibitory effect of esculetin against the AChE activity [Figure 6].

IL-6, IL-1β, and TNF-α are important pro-inflammatory mediators that play an essential function in the inflammatory reactions. They can provoke the over-accumulation of hyperoxide and deplete the cholinergic activity via elevated AChE activity to speed up the development of neurodegenerative diseases. Increased cytokine levels in the brain cause pathological changes such as inflammation, nitrification, and oxidation and disturb nerve homeostasis.[57] According to our results, there was a drastic elevation in the level of IL-6, IL-1β, and TNF-α on the brain tissues of D-gal-induced AD animals. Interestingly, esculetin significantly reduced the level of IL-6, IL-1β, and TNF-α in the brain tissues of AD rats, which demonstrates its anti-inflammatory property [Figure 5].

D-gal drastically elevates the level of several oxidative biomarkers such as AGEs (an oxidative marker of protein), OhdG (an oxidative marker of DNA), and 8-iso-PGF.[58–60] In this study, we obtained similar results. Interestingly, esculetin significantly reduced the levels of AGEs, OhdG, and 8-iso-PGF in D-gal-induced AD animals [Figure 4]. These results provide evidence of the antioxidant potential of esculetin.

CONCLUSION

In conclusion, esculetin attenuated oxidative damage and neuroinflammation by increasing the neuronal antioxidant enzyme activities, decreasing oxidative damage biomarkers, reducing pro-inflammatory cytokines' levels, and triggering cholinergic mechanisms in D-gal-induced AD animals. Esculetin can be considered as a remedial agent to treat AD in the future. However, we recommend further studies to understand the underlying mechanisms of the mode of action of esculetin.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Prince M, Wimo A, Guerchet M, Ali GC, Wu Y, Prina AM. World Alzheimer Report 2015: The global impact of dementia. An analysis of prevalence, incidence, costs and trends. Alzheimer's Disease International, London, 2015.
2. Alzheimer's Association. Alzheimer's disease facts and figures. Alzheimer's Dement 2019;15:321-87.
3. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem 2010;345:91-104.
4. Schrag M, Mueller C, Zabel M, Crofton A, Kirsch WM, Ghribi O, *et al.* Oxidative stress in blood in Alzheimer's disease and mild cognitive impairment: A meta-analysis. Neurobiol Dis 2013;59:100-10.
5. Flannery PJ, Trushina E. Mitochondrial dynamics and transport in Alzheimer's disease. Mol Cell Neurosci 2019;98:109-20.
6. Morgan AR, Touchard S, Leckey C, O'Hagan C, Nevado-Holgado AJ, Barkhof F, *et al.* Inflammatory biomarkers in Alzheimer's disease plasma. Alzheimers Dement 2019;15:776-87.
7. Ozben T, Ozben S. Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease. Clin Biochem 2019;72:87-9.
8. Peña-Bautista C, Baquero M, Vento M, Cháfer-Pericás C. Free radicals in Alzheimer's disease: Lipid peroxidation biomarkers. Clin Chim Acta 2019;491:85-90.
9. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement 2018;4:575-90.
10. Zhang D, Wang X, Li R, Wang L, Zhou Z, Fu Q, *et al.* Extract of the aerial part of Polygala tenuifolia attenuates d-galactose/NaNO2-induced learning and memory impairment in mice. Planta Med 2020;86:1389-99.
11. Ho SC, Liu JH, Wu RY. Establishment of the mimetic aging effect in mice caused by D-galactose. Biogerontology 2003;4:15-8.
12. Wei H, Li L, Song Q, Ai H, Chu J, Li W. Behavioural study of the D-galactose induced aging model in C57BL/6J mice. Behav Brain Res 2005;157:245-51.
13. Lei H, Wang B, Li WP, Yang Y, Zhou AW, Chen MZ. Anti-aging effect of astragalosides and its mechanism of action. Acta Pharmacol Sin 2003;24:230-4.
14. Tian J, Ishibashi K, Ishibashi K, Reiser K, Grebe R, Biswal S, *et al.* Advanced glycation endproduct-induced aging of the retinal pigment epithelium and choroid: A comprehensive transcriptional response. Proc Natl Acad Sci U S A 2005;102:11846-51.
15. Gao J, Zhou R, You XT, Luo F, He H, Chang XY, *et al.* Salidroside suppresses inflammation in a D-galactose-induced rat model of Alzheimer's disease via SIRT1/NF-kappaB pathway. Metab Brain Dis 2016;31:771-8.
16. Gao J, He H, Jiang W, Chang X, Zhu L, Luo F, *et al.* Salidroside ameliorates cognitive impairment in a d-galactose-induced rat model of Alzheimer's disease. Behav Brain Res 2015;293:27-33.
17. Subramaniam SR, Ellis EM. Esculetin-induced protection of human hepatoma HepG2 cells against hydrogen peroxide is associated with the Nrf2-dependent induction of the NAD (P) H: Quinone oxidoreductase 1 gene. Toxicol Appl Pharmacol 2011;250:130-6.
18. Ozal SA, Turkekul K, Gurlu V, Guclu H, Erdogan S. Esculetin protects human retinal pigment epithelial cells from lipopolysaccharide-induced inflammation and cell death. Curr Eye Res 2018;43:1169-76.
19. Xu F, Li X, Liu L, Xiao X, Zhang L, Zhang S, *et al.* Attenuation of doxorubicin-induced cardiotoxicity by esculetin through modulation of Bmi-1 expression. Exp Ther Med 2017;14:2216-20.
20. Zhen AX, Piao MJ, Kang KA, Fernando PDSM, Kang HK, Koh YS, *et al.* Esculetin prevents the induction of matrix metalloproteinase-1 by hydrogen peroxide in skin keratinocytes. J Cancer Prev 2019;24:123-8.
21. Jeong NH, Yang EJ, Jin M, Lee JY, Choi YA, Park PH, *et al.* Esculetin from Fraxinus rhynchophylla attenuates atopic skin inflammation by inhibiting the expression of inflammatory cytokines. Int Immunopharmacol 2018;59:209-16.
22. Liang C, Ju W, Pei S, Tang Y, Xiao Y. Pharmacological activities and synthesis of esculetin and its derivatives: A mini-review. Molecules 2017;22:387.
23. Kadakol A, Sharma N, Kulkarni YA, Gaikwad AB. Esculetin: A phytochemical endeavor fortifying effect against non-communicable diseases. Biomed Pharmacother 2016;84:1442-8.
24. Rzodkiewicz P, Gąsińska E, Gajewski M, Bujalska-Zadrożny M, Szukiewicz D, Maśliński S. Esculetin reduces leukotriene B4 level in plasma of rats with adjuvant-induced arthritis. Reumatologia 2016;54:161-4.
25. Chen Y, Zhang Q, Liu H, Lu C, Liang CL, Qiu F, *et al.* Esculetin ameliorates psoriasis-like skin disease in mice by inducing CD4+Foxp3+regulatory T cells. Front Immunol 2018;9:2092.
26. Lee HC, Liu FC, Tsai CN, Chou AH, Liao CC, Yu HP. Esculetin ameliorates lipopolysaccharide-induced acute lung injury in mice via modulation of the AKT/ERK/NF-κB and RORγt/IL-17 pathways. Inflammation 2020;43:962-74.
27. Gong YS, Guo J, Hu K, Gao YQ, Xie BJ, Sun ZD, *et al.* Ameliorative effect of lotus seedpod proanthocyanidins on cognitive impairment and brain aging induced by D-galactose. Exp Gerontol 2016;74:21-8.
28. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11:47-60.
29. Jordan WH, Young JK, Hyten MJ, Hall DG. Preparation and analysis of the central nervous system. Toxicol Pathol 2011;39:58-65.
30. Tsuno N. Donepezil in the treatment of patients with Alzheimer's disease. Expert Rev Neurother 2009;9:591-8.
31. Terry AV, Buccafusco JJ. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: Recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 2003;306:821-7.
32. Liu L, Lu Y, Kong H, Li L, Marshall C, Xiao M, *et al.* Aquaporin–4 deficiency exacerbates brain

oxidative damage and memory deficits induced by long-term ovarian hormone deprivation and D-galactose injection. Int J Neuropsychopharmacol 2012;15:55-68.

33. Krivinko JM, Koppel J, Savonenko A, Sweet RA. Animal models of psychosis in Alzheimer disease. Am J Geriatr Psychiatry 2020;28:1-19.
34. Yang HG, Qu Z, Zhang JZ, Huo LQ, Gao J, Gao WY. Ferulic acid ameliorates memory impairment in d-galactose-induced aging mouse model. Int J Food Sci Nutr 2016;67:806-17.
35. Cui X, Zuo P, Zhang Q, Li X, Hu Y, Long J, *et al*. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: Protective effects of R-alpha-lipoic acid. J Neurosci Res 2006;84:647-54.
36. Mariani E, Polidori MC, Cherubini A, Mecocci P. Oxidative stress in brain aging, neurodegenerative and vascular diseases: An overview. J Chromatogr B Analyt Technol Biomed Life Sci 2005;827:65-75.
37. Zaki HF, Abd-El-Fattah MA, Attia AS. Naringenin protects against scopolamine-induced dementia in rats. Bull Fac Pharm Cairo Univ 2014;52:15-25.
38. Baydar T, Papp A, Aydin A, Nagymajtenyi L, Schulz H, Isimer A, *et al*. Accumulation of aluminum in rat brain: Does it lead to behavioral and electrophysiological changes? Biol Trace Elem Res 2003;92:231-44.
39. Yang WN, Han H, Hu XD, Feng GF, Qian YH. The effects of perindopril on cognitive impairment induced by D-galactose and aluminum trichloride via inhibition of acetylcholinesterase activity and oxidative stress. Pharmacol Biochem Behav 2013;114-115:31-6.
40. Kim GH, Kim JE, Rhie SJ, Yoon S. The role of oxidative stress in neurodegenerative diseases. Exp Neurobiol 2015;24:325-40.
41. Khan MB, Khan MM, Khan A, Ahmed ME, Ishrat T, Tabassum R, *et al*. Naringenin ameliorates Alzheimer's disease (AD)-type neurodegeneration with cognitive impairment (AD-TNDCI) caused by the intracerebroventricular-streptozotocin in rat model. Neurochem Int 2012;61:1081-93.
42. Patel NV, Gordon MN, Connor KE, Good RA, Engelman RW, Mason J, *et al*. Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. Neurobiol Aging 2005;26:995-1000.
43. Mattson MP. Pathways towards and away from Alzheimer's disease. Nature 2004;430:631-9.
44. Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. Nucleic Acids Res 2007;35:7497-504.
45. Patel M. Targeting oxidative stress in central nervous system disorders. Trends Pharmacol Sci 2016;37:768-78.
46. Ikram M, Muhammad T, Rehman SU, Khan A, Jo MG, Ali T, *et al*. Hesperetin confers neuroprotection by regulating Nrf2/TLR4/NF-κB signaling in an Aβ mouse model. Mol Neurobiol 2019;56:6293-309.
47. Xue M, Rabbani N, Momiji H, Imbasi P, Anwar MM, Kitteringham N, *et al*. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. Biochem J 2012;443:213-22.
48. Johnson JA, Johnson DA, Kraft AD, Calkins MJ, Jakel RJ, Vargas MR, *et al*. The Nrf2-ARE pathway: An indicator and modulator of oxidative stress in neurodegeneration. Ann N Y Acad Sci 2008;1147:61-9.
49. Poon HF, Calabrese V, Scapagnini G, Butterfield DA. Free radicals: Key to brain aging and heme oxygenase as a cellular response to oxidative stress. J Gerontol A Biol Sci Med Sci 2004;59:478-93.
50. Naidu RN, Shankar B, Dsouza U. Effect of long-term administration of aluminium chloride on oxidative stress and acetylcholinesterase activity in rat brains. Int J Pharm Biol Sci 2013;3:616-22.
51. Chang L, Liu X, Liu J, Li H, Yang Y, Liu J, *et al*. D-galactose induces a mitochondrial complex I deficiency in mouse skeletal muscle: Potential benefits of nutrient combination in ameliorating muscle impairment. J Med Food 2014;17:357-64.
52. McKeever PM, Kim T, Hesketh AR, MacNair L, Miletic D, Favrin G, *et al*. Cholinergic neuron gene expression differences captured by translational profiling in a mouse model of Alzheimer's disease. Neurobiol Aging 2017;57:104-19.
53. Klaassens BL, van Gerven JMA, Klaassen ES, van der Grond J, Rombouts SARB. Cholinergic and serotonergic modulation of resting state functional brain connectivity in Alzheimer's disease. Neuroimage 2019;199:143-52.
54. Haider S, Liaquat L, Shahzad S, Sadir S, Madiha S, Batool Z, *et al*. A high dose of short term exogenous d-galactose administration in young male rats produces symptoms simulating the natural aging process. Life Sci 2015;124:110-9.
55. Xian YF, Su ZR, Chen JN, Lai XP, Mao QQ, Cheng CHK, *et al*. Isorhynchophylline improves learning and memory impairments induced by D-galactose in mice. Neurochem Int 2014;76:42-9.
56. Xian YF, Lin ZX, Zhao M, Mao QQ, Ip SP, Che CT. Uncaria rhynchophylla Ameliorates Cognitive Deficits Induced by D-galactose in Mice. Planta Med 2011;7:1977-83.
57. Bastin C, Delhaye E, Moulin C, Barbeau EJ. Novelty processing and memory impairment in in Alzheimer's disease: A review. Neurosci Biobehav Rev 2019;100:237-49.
58. Salahuddin P, Rabbani G, Khan RH. The role of advanced glycation end products in various types of neurodegenerative disease: A therapeutic approach. Cell Mol Biol Lett 2014;19:407-37.
59. Gackowski D, Rozalski R, Siomek A, Dziaman T, Nicpon K, Klimarczyk M, *et al*. Oxidative stress and oxidative DNA damage is characteristic for mixed Alzheimer disease/vascular dementia. J Neurol Sci 2008;266:57-62.
60. Waddington E, Croft K, Clarnette R, Mori T, Martins R. Plasma F-2-isoprostane levels are increased in Alzheimer's disease: Evidence of increased oxidative stress *in vivo*. Alzheimers Rep 1999;2:277-82.