Antioxidant, anti-inflammatory, and neuroprotective role of pterostilbene in attenuating Alzheimer-like pathology in AlCl3-induced rats' model of Alzheimer's disease

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Abstract:

Background: Alzheimer's disease (AD) is a degenerative neurological ailment marked by the accumulation of aberrant protein deposits in the brain, including beta-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) formed from tau protein.

Objectives: to evaluate the potential preventive effects of pterostilbene against aluminum chloride-induced Alzheimer's disease in rats, examining its influence on oxidative and inflammatory markers and histopathological changes alterations.

Method: involved the random creation of five groups, each consisting of eight rats. Induction of Alzheimer's disease was achieved using $AlCl_3$ (70 mg/kg, intraperitoneally) for 30 days, followed by treatment with PTE (50 and 100 mg/kg, orally) or donepezil (0.1 mg/kg, orally) for 14 days, except for the control group, which received normal saline for the initial 30 days, followed by the vehicle alone. The open field test was employed for the neurobehavioral assessment of the rats. A biochemical examination was subsequently conducted utilizing ELISA kits to demonstrate the impact of PTE on the levels of 8-Epi-Prostaglandin F2α (8-epi-PGF2α), IL-1β, and TNF-α, and a colorimetric kit to demonstrate the impact of PTE on the total antioxidant capacity (TAOC). Additional validations were conducted through histopathological evaluation of the cortex by Congo red stain.

Result: Exposure to $AlCl_3$ significantly elevated 8-epi-PGF2α, IL-1β and TNF-α while markedly decreasing TAOC. These results were reversed in PTE-treated rats. Histopathological assessment of the cortex in rat brains produced by $AlCl_3$ demonstrated pathological changes. such pathology was not present in rats administered PTE.

Conclusion: A conducted study showed the anti-AD activity of PTE by reducing oxidative stress and inflammation, and amyloidosis, possibly by decreasing 8-epi-PGF2α, IL-1β and TNF-α and increasing TAOC.

Keywords: Alzheimer's disease, Aluminum chloride, Pterostilbene, open field test, oxidative stress, inflammatory biomarkers.

الدور المضاد للأكسدة والمضاد للالتهاب والعصبي الوقائي للتيروستلبين في التخفيف من مرض ألزهايمر الشبيه بالخرف في نموذج الجرذان المستحث بكلوريد الألمنيوم.

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الخلاصة

الخلفية :يُعدّ مرض ألزهايمر (AD) اضطرابًا عصبيًا تنكسيًا يتميز بتراكم ترسّبات بروتينية شاذة في الدماغ، بما في ذلك لويحات بيتا-أميلويد (Aβ) والتشابكات الليفية العصبية (NFTs) المتكوّنة من بروتين تاو.

الأهداف :تقييم التأثيرات الوقائية المحتملة لمركب التروستلبين ضد مرض ألزهايمر المُستَحدث بواسطة كلوريد الألمنيوم في الجرذان، من خلال دراسة تأثيره على مؤشرات الإجهاد التأكسدي والالتهاب والتغيرات النسيجية المرضية.

المنهجية :شملت الدراسة التوزيع العشوائي لخمسة مجموعات، تضم كل منها ثمانية جرذان. تم استحداث مرض ألزهايمر باستخدام $AlCl_3$بجرعة (70 ملغم/كغم، داخل الصفاق) لمدة 30 يومًا، تلاها العلاج إما بالتروستلبين (50 و100 ملغم/كغم، فمويًا) أو دونيبيزيل (0.1 ملغم/كغم، فمويًا) لمدة 14 يومًا، باستثناء مجموعة السيطرة التي تلقت محلولًا ملحيًا عاديًا خلال الثلاثين يومًا الأولى تلاه المحلول المذاب فيه الادوية فقط. استُخدم اختبار الحقل المفتوح لتقييم الوظائف السلوكية العصبية للجرذان. وأُجري الفحص البيوكيميائي باستخدام أطقم ELISA لقياس تأثير التروستلبين على مستويات 8-إيبي-بروستاغلاندينF2α (8-epi-PGF2α)، والإنترلوكين-1β (IL-1β)، وعامل نخر الفا (TNF-α)، إضافةً إلى استخدام كيت لوني لتحديد السعة الكلية المضادة للأكسدة (TAOC).كما أُجري التقييم النسيجي المرضي لقشرة الدماغ باستخدام صبغة الكونغو الأحمر.

النتائج :أدى التعرض لكلوريد الألمنيوم إلى ارتفاع ملحوظ في مستويات 8-epi-PGF2α، وIL-1β، وTNF-α، مع انخفاض كبير في السعة الكلية المضادة للأكسدة. وقد انعكست هذه النتائج في الجرذان المعالجة بالتروستلبين. أما الفحص النسيجي المرضي للقشرة الدماغية فقد أظهر تغيرات مرضية ناجمة عن $AlCl_3$ لم تُلاحظ في الجرذان التي أعطيت التروستلبين.

الاستنتاج :أظهرت الدراسة المُجراة الفعالية المضادة لمرض ألزهايمر لمركب التروستلبين من خلال تقليل الإجهاد التأكسدي والالتهاب والتنشؤ النشواني، وربما عبر خفض مستويات 8 epi-PGF2α- وIL-1β وTNF-αوزيادة السعة الكلية المضادة للأكسدة.

الكلمات المفتاحية :مرض ألزهايمر، كلوريد الألمنيوم، التروستلبين، اختبار الحقل المفتوح، الإجهاد التأكسدي، المؤشرات الالتهابية.

INTRODUCTION

Alzheimer's disease (AD), the predominant etiology of dementia comprising 60% to 80% of instances, is a degenerative neurological ailment marked by the accumulation of aberrant protein deposits in the brain, including beta-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) formed from tau protein. These deposits interfere with cellular processes, hinder neuronal connections, and lead to the deterioration and demise of brain cells, culminating in impairment of memory, cognitive decline, and alterations in behavior(1,2).

The incidence of all-cause dementia is projected to rise from 50 million individuals in 2010 to 113 million by 2050 globally. Despite the rising frequency of dementia in both high-income and middle/low-income nations over the past 50 years due to greater life expectancy, the incidence of dementia has somewhat declined in several high-income countries, including the USA, UK, and France(3).Aging is the predominant risk factor for Alzheimer's disease, with the prevalence of all dementias doubling every 6.3 years, increasing from 3.9 per 1000 individuals aged 60–90 to 104.8 per 1000 those over age 90. The prevalence is estimated at 10% for persons aged over 65 years and 40% for those aged over 80 years. The escalating personal and financial burdens necessitate efficient pre-clinical diagnostics and interventions to impede disease

development prior to the emergence of symptoms(4,5).

Multiple variables contribute to the complexity of Alzheimer's disease. still don't know much about the specific pathophysiology of AD because human brains are so complicated and because there aren't enough good animal models and research tools. Several theories about Alzheimer's disease (AD) have been put up, such as amyloidβ (Aβ), Tau, damage to cholinergic neurons and oxidative stress, inflammation, and so on. Accordingly, these theories have been the basis for several attempts to create anti-AD medications(6).

Reactive oxygen species (ROS) are chemicals generated as byproducts of normal cellular metabolism that have a role in the etiology of Alzheimer's disease (AD). Oxidative stress arises from the disparity between the levels of reactive oxygen species (ROS) and the cellular capacity for neutralization. Multiple studies have shown that heightened oxidative stress plays a significant role in initiating pathogenic alterations throughout the early and asymptomatic phases of Alzheimer's disease(7). The brain's structure, rapid metabolism, and elevated lipid levels render it susceptible to the detrimental effects of oxidative stress. An investigation using mice deficient in Cu/Zn superoxide dismutase (SOD) has demonstrated that elevated oxidative stress leads to a deterioration of cognitive function similar to that observed in aged mouse models. Furthermore, decreased levels of antioxidant enzymes have been observed in animal models and individuals with Alzheimer's disease. Consequently, regulating oxidative stress through the use of antioxidants may represent a viable approach for addressing this condition(8).The aggregate cores of senility plaques in Alzheimer's disease include increased amounts of transition metal ions, specifically copper, iron, and zinc. These ions can facilitate the reduction of oxygen to hydrogen peroxide, and subsequently, hydrogen peroxide to the hydroxyl radical. This radical may cause considerable oxidative damage(9,10).

Neuroinflammation is a significant factor in the etiology of Alzheimer's disease, both initiating and exacerbating the deposition of Aβ and tau proteins. The neuroinflammatory response in Alzheimer's disease involves a multifaceted reaction characterized by the recruitment of peripheral immune cells such as leukocytes and T cells, activation of glial cells, induction of intracellular signaling pathways, and the generation of inflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), tumor necrosis factor-α (TNF-α), interferons (IFN), and interleukin-12 (IL-12). These cytokines are increased in characteristic Alzheimer's disease locations, leading to neuronal malfunction or death(11). Aluminum chloride ($AlCl_3$) was widely employed to induce dementia in many animal models. Aluminum is a known neurotoxin associated with the degenerative evolution of several neurological disorders. Aluminum can function as a cross-linker of amyloid β-protein, leading to oligomerization and thereby inducing neurotoxicity. Moreover, it has been previously documented that prolonged exposure to $AlCl_3$ may induce dementia in Wistar rats(12).

Different doses of $AlCl3_3 \cdot 6H2O$ have been used in animals in earlier research to mimic Alzheimer's disease phases. According to Ahmed et al. (2022), male rats given a dose of 70 mg/kg/day to induce Alzheimer's disease showed severe learning and memory impairments, poor behavioral performance, and abnormalities in their biochemistry and histology(13). After giving rats intraperitoneal injections of $AlCl_3$ and D-gal (150 mg/kg and 300 mg/kg, respectively) for a week, Haider et al. (2020) observed biochemical and histological alterations in

addition to the onset of symptoms resembling Alzheimer's disease(14).

Although the illness was diagnosed more than a century ago, efforts are presently focused on discovering novel chemical agents (i.e., natural antioxidants) that target specific pathways to impede the disease's progression(15).Pterostilbene, a phenolic molecule similar to resveratrol, is found in sandalwood, grapes, and blueberries. It is known for its antioxidant and anti-inflammatory properties, particularly in aging brains. In vitro, it has been found to induce apoptosis in cancer cells more effectively than resveratrol. Pterostilbene also has significant agonistic properties on the peroxisome proliferator-activated receptor (PPAR) alpha, a receptor complex linked to fatty acid metabolism, inflammation, and oxidative stress regulation. However, there is limited understanding of its impact on neural and cognitive functions and potential protective benefits against age-related diseases(16,17).

Pterostilbene, a low-molecular-weight and highly lipid-soluble compound, is widely distributed across various tissues, with a distribution volume exceeding that of body water. It is predominantly found in tissues, not blood, and its biological activity is evident even at low blood or plasma levels. The highest concentration of pterostilbene was found in brain tissue, suggesting it can easily cross the blood-brain barrier(18).

The objective of this presented study was to evaluate the potential preventive effects of pterostilbene against aluminum chloride-induced Alzheimer's disease in rats, examining its influence on oxidative markers and histopathological alterations.

MATERIALS AND METHODS

Chemicals and reagents

Sigma-Aldrich (USA) provided pterostilbene, DMSO, Congo red stain, and 10% formalin. Merck (Germany) provided aluminum chloride hexahydrate ($AlCl_3 \cdot 6H2O$). Donepezil was purchased from Wuhan Lullaby Biotechnology (China). Ketamine was purchased from Bioveta (Czech Republic). Pioneer (Iraq) supplied normal saline. The paraffin wax came from Dalian (chain). Tween 80 was bought from Croda International (British). Alfasan (Netherlands) supplied xylazine. The ELISA and colorimetric kits were purchased from Elabscience Biotechnology Inc. (USA).

Animals care and ethical approval

The Iraqi Center for Genetics and Cancer Research's Animal House provided forty male albino Wistar rats, ages 8–10 weeks, weighing 200–250 grams. In order to circumvent the hormonal and physiological fluctuations linked to the estrous cycle in female rats, male rats were employed in this investigation. Inside, the animals were kept in the animal house at the College of Pharmacy/Mustansiriyah University in plastic cages with sufficient ventilation. Woodchip bedding was included with each cage, which had dimensions of 20 x 25 x 35 cm. The animals were housed in conventional settings, which included a 12-hour light-dark cycle, a temperature of 25 ± 2°C, and a humidity level of 30% to 40%(19). On October 10, 2024, the study was approved by Mustansiriyah University's College of Pharmacy's animal ethical committee under Approval No. 41.

Experimental Design

The experimental design **(Fig. 1)** involved the random creation of five groups, each consisting of eight rats. Induction of Alzheimer's disease was achieved using $AlCl_3$ (70 mg/kg, intraperitoneally) for 30 days, followed by treatment with PTE (50 and 100 mg/kg, orally) or donepezil (0.1 mg/kg, orally) for 14 days, except for the control group, which received normal saline

for the initial 30 days, followed by the vehicle alone. On the 44th day the An open field activity monitoring test was applied and On the 45th day, rats were terminated via intraperitoneal injections of ketamine and xylazine at doses of 100 mg/kg and 10 mg/kg, respectively(20). Subsequently, the rat brains were extracted and meticulously washed with distilled water. The brain was divided into two hemispheres. One specimen was stored in 10% neutral formalin for histological analysis, while the other was weighed and homogenized with phosphate-buffered saline (PBS) using a pre-chilled micro-homogenizer for evaluation of oxidative markers (8-Epi-Prostaglandin F2 Alpha (8-epi-PGF2α), Total Antioxidant Capacity (TAOC)) and inflammatory markers (IL-1β, TNF-α). The material was subsequently spun for 20 minutes at 20,000 g using a cooled centrifuge. The supernatant is then collected without delay(21).

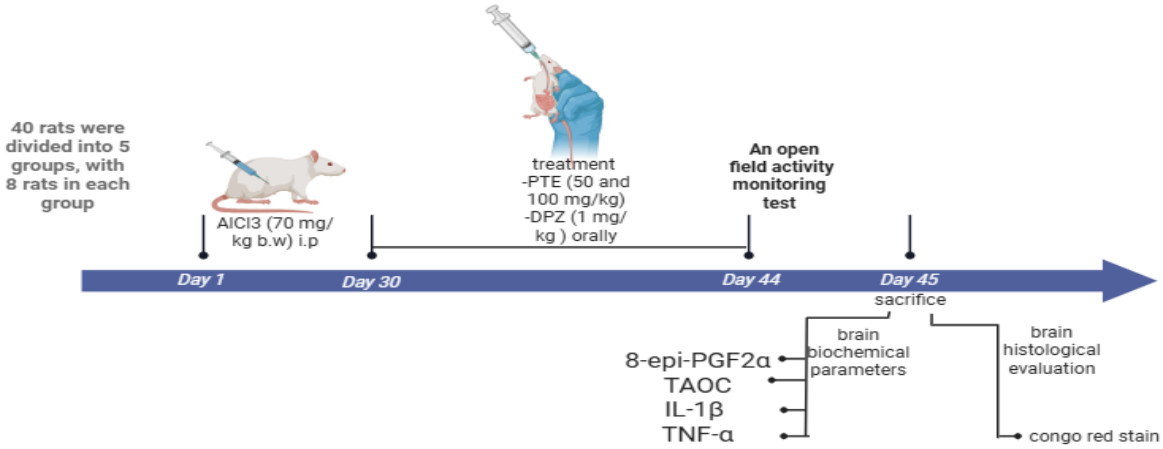

Figure (1) Representative illustration depicting the induction and treatment protocol in rats:8-epi-PGF2α=8-Epi-Prostaglandin F2 Alpha, TAOC=Total antioxidant capacity, CTRL= control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene

Behavioral analysis

An open field activity monitoring test

This test was conducted to evaluate the movement and behavioral patterns of the rats on (day 44). Observations were documented in a locally made wooden apparatus partitioned into 25 squares (5x5). The animal was positioned in one corner of the chamber, and its behavior was monitored for 5 minutes. The test quantifies (i) the number of squares explored, (ii) the total time spent immobile (in seconds), (iii) instances of rearing, and (iv) the number of faecal pellets produced. (22,23).

Biochemical parameters

The amounts of proteins in brain tissue homogenates were assessed utilizing the enzyme-linked immunosorbent assay (ELISA) and colorimetric method, adhering to the manufacturer's procedure to compare biomarker levels among groups. The ELISA kits (8-epi-PGF2α, IL-1β, TNF-α) utilize a micro-ELISA plate that has been previously coated with a rat antibody, employing the Sandwich-ELISA method. After standards or samples are introduced, biotinylated detection antibody and Avidin-Horseradish Peroxidase (HRP) are added. Following incubation, an acidic stop solution is

introduced, and the unbound streptavidin-HRP is eliminated. At 450 nm, the absorbance is then measured(24). While the colorimetric kit (TAOC) utilizes a standard technique based on single-electron transfer that quantifies the reduction of ferric ion (Fe3+)-ligand complexes to the bright-colored ferrous complex (Fe2+), facilitated by antioxidants under acidic conditions. The total antioxidant capacity (T-AOC) is determined by measuring absorbance at 520 nm(25).Every sample and standard has undergone double testing

$$Fe^{3+} + \text{Antioxidant} \rightarrow Fe^{2+}$$
$$Fe^{2+} + 3(1,10\text{-phenanthroline}) \rightarrow [Fe(phen)_3]^{2} \text{ (colored)}$$

Histopathological examination

For the purpose of histological examination, half-brain tissue samples were fixated for twenty-four hours at room temperature in 10% buffered neutral formalin. To avoid undue hardness, the tissue specimens were immersed in ethanol solutions that were getting progressively stronger after fixation. Later, after labeling, the samples were placed in paraffin wax, which was melted at 60 ˚C for 15 minutes to form blocks. The next step was to slice the tissue at a 5-micrometre thickness using a semi-automated rotary microtome. After being stained with Congo red and put on clean glass slides, the slices were viewed under microscopes at different magnifications(26,27)A proficient pathologist diligently assessed and documented pathological abnormalities, including secondary amyloidosis. A semi-quantitative scoring system (ordinal scale) was employed: the severity of cellular alterations was defined as (0, 1, 2, 3), denoting absence, mild, moderate, and severe, respectively(28).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 10. The data were examined with a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test to assess the significance of differences between means. P-value ≤0.05 was deemed statistically significant.

The histopathological scoring system was based on mean ranks, using the non-parametric Kruskal-Wallis one-way ANOVA followed by Dunn's post hoc test, which was used to identify pairwise group differences. Significant differences were identified; the histological data, represented as scores categorized into four levels, do not follow a normal distribution, as tested by the Shapiro-Wilk test, making the non-parametric test an appropriate tool.

RESULTS

Effect of pterostilbene on memory and behavioral performance tests

An open field activity monitoring test

Number of squares crossed

In comparison to the induction ($AlCl_3$) group (20.000±1.414), the number of squares crossed was significantly higher (P-value ≤0.05) in the control (CTRL), DPZ, PTE 50 mg/kg, and PTE 100 mg/kg groups (49.833±1.471, 34.833±1.471, 36.000±1.549, and 45.000±1.788, respectively). The number of squares crossed exhibited a statistically significant decrease (P-value < 0.05) in the PTE 50 mg/kg group compared to the control and the PTE 100 mg/kg group while not demonstrating a statistically significant difference (P > 0.05) when compared to those receiving DPZ, as seen in **(Fig. 2)**.

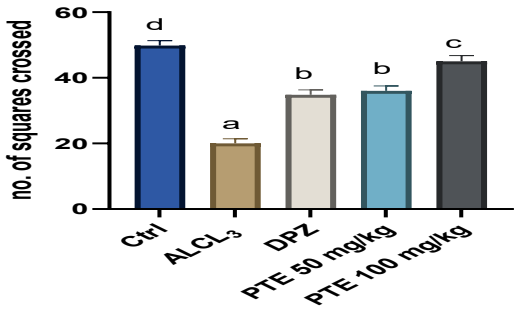

Figure 2: Effect of pterostilbene number of squares crossed among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=6): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups ($p < 0.05$).

Time spent immobile

Compared to the induction ($AlCl_3$) group (299.167±11.583), the time of immobility was significantly lower (P-value ≤0.05) in the control (CTRL), DPZ, PTE 50 mg/kg, and PTE 100 mg/kg groups (128.500±5.357, 186.333±3.669, 184.000±3.162, and 139.833±3.970, respectively). The immobility time showed a statistically significant increase (P-value < 0.05) in the PTE 50 mg/kg group compared to the PTE 100 mg/kg group. In contrast, no statistically significant change (P > 0.05) was seen compared to the DPZ group, as illustrated in **(Fig. 3)**.

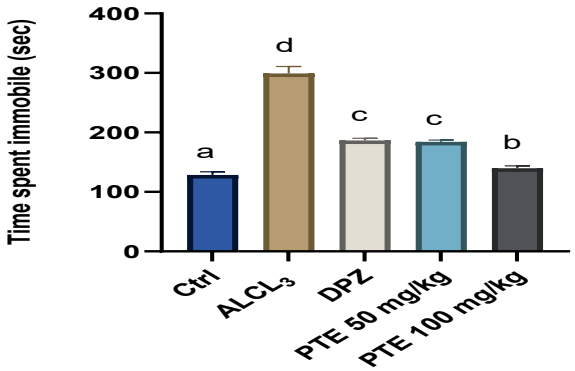

Figure 3: Effect of pterostilbene on time spent immobile among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=6): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups ($p < 0.05$).

Number of rearing

The induction ($AlCl_3$), DPZ, PTE 50 mg/kg, and PTE 100 mg/kg groups (5.000±1.414, 5.500±1.378, 4.833±1.169, and 4.666±1.032, respectively) exhibited no statistically significant difference ($P > 0.05$) in Number of rearing when compared to the control (CTRL) group (4.666±1.032), as illustrated in **(Fig. 4)**.

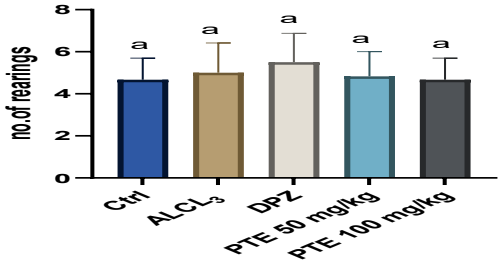

Figure 4: Effect of pterostilbene on the number of rearing among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=6): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups ($p < 0.05$).

Number of fecal pellets

The induction ($AlCl_3$), DPZ, PTE 50 mg/kg, and PTE 100 mg/kg groups (1.500±0.547, 1.500±0.836, 1.166±0.408, and 1.500±0.547, respectively) demonstrated no statistically significant difference ($P > 0.05$) in the number of fecal pellets compared to the control (CTRL) group (1.166±0.408), as depicted in **(Fig. 5)**.

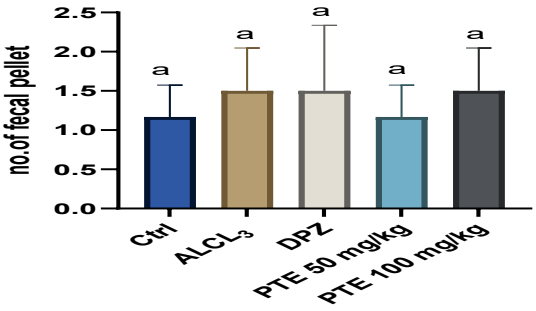

Figure 5: Effect of pterostilbene on the number of fecal pellets among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=6): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups ($p < 0.05$).

Effect of pterostilbene on oxidative markers

8-Epi-Prostaglandin F2 Alpha (8-epi-PGF2α)

The conducted study demonstrated that 8-epi-PGF2α levels were significantly lower (P-value ≤0.05) in the control (CTRL), DPZ, and PTE 50 mg/kg and PTE 100 mg/kg groups (174.031±13.265, 238.942±33.866, 294.241±6.832, and 191.586±18.585pg/mL, respectively) compared to the induction ($AlCl_3$) group (420.000±10.766pg/mL). The PTE 100 mg/kg group exhibited a significant decrease (P-value ≤0.05) compared to the DPZ and PTE 50 mg/kg groups. Upon comparison of the PTE 100 mg/kg groups with the CTRL group, no statistically significant differences in 8-epi-PGF2α levels were observed (P > 0.05); this dose can approach the control group. As shown in the **(Fig. 6)**.

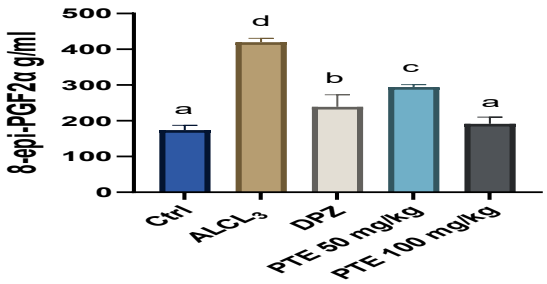

Figure 6: Effect of pterostilbene on 8-Epi-Prostaglandin F2 Alpha (8-epi-PGF2α) among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=8): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups (p < 0.05).

Total antioxidant capacity (TAOC)

The conducted study demonstrated that TAOC levels were significantly elevated (P-value ≤0.05) in control (CTRL), DPZ, and PTE 100 mg/kg groups (394.244±10.155, 356.234±45.709, 342.818±10.124pg/mL, respectively) in comparison to the induction ($AlCl_3$) group (261.719±16.495pg/mL). However, when comparing the PTE 50 mg/kg group (278.622±5.586) to the $AlCl_3$ group, no statistically significant differences in TAOC levels were observed (P > 0.05). The PTE 50 mg/kg group exhibited a substantial reduction (P-value ≤0.05) compared to the CTRL, DPZ, and PTE 100 mg/kg groups. In the comparison between the PTE 100 mg/kg groups and the DPZ group, no statistically significant differences in TAOC levels were observed (P > 0.05), as shown in **(Fig. 7)**.

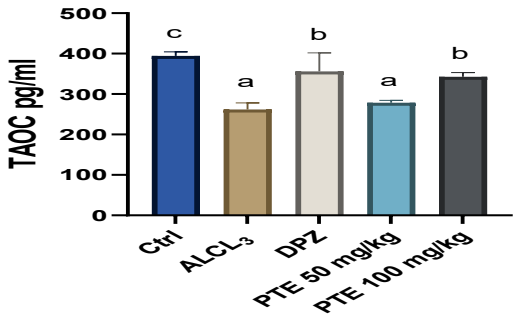

Figure 7: Effect of pterostilbene on total antioxidant capacity (TAOC) among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=8): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups ($p < 0.05$).

Effect of pterostilbene on inflammatory markers

Interleukin 1Beta (IL-1β)

It's clear **(Fig. 8)** shows that compared to the induction ($AlCl_3$) group (380.540±8.51878pg/mL), the levels of IL-1β were significantly lower (P-value ≤0.05) in the control (CTRL), DPZ, PTE 50 mg/kg, and PTE 100 mg/kg groups (169.946±9.811, 170.158±30.688, 287.927±11.753, and 183.476±19.600pg/mL, respectively). Nonetheless, IL-1β levels did not differ significantly (P > 0.05) between the CTRL group and those treated with DPZ or PTE 100 mg/kg. The PTE 100 mg/kg group had a statistically significant reduction (P-value ≤0.05) when contrasted with the PTE 50 mg/kg group. Nonetheless, when contrasted with the DPZ group, no statistically significant difference was found (P > 0.05).

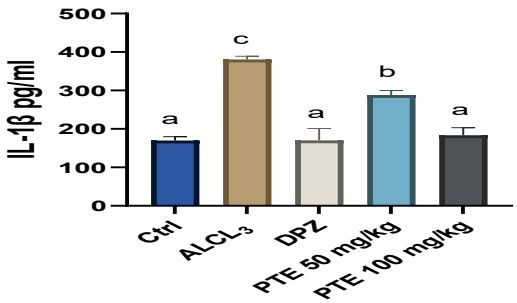

Figure 8: Effect of pterostilbene on interleukin 1Beta (IL-1β) among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=8): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups ($p < 0.05$).

Tumor necrosis factor alpha (TNF-α)

It's evident in comparison to the induction group ($AlCl_3$) (367.459±18.608 pg/mL), the control group (CTRL), DPZ group, PTE 50 mg/kg group, and PTE 100 mg/kg group had significantly lower levels of TNF-α (P-value ≤0.05) (131.505±35.405,180.212 ±12.018, 299.505±16.049, and 193.359±48.333 pg/mL), respectively. Compared to the PTE 50 mg/kg group, the PTE 100 mg/kg group showed a statistically significant decrease (P-value ≤0.05). However, no significant change was compared to the DPZ group (P > 0.05). As shown in the **(Fig. 9)**.

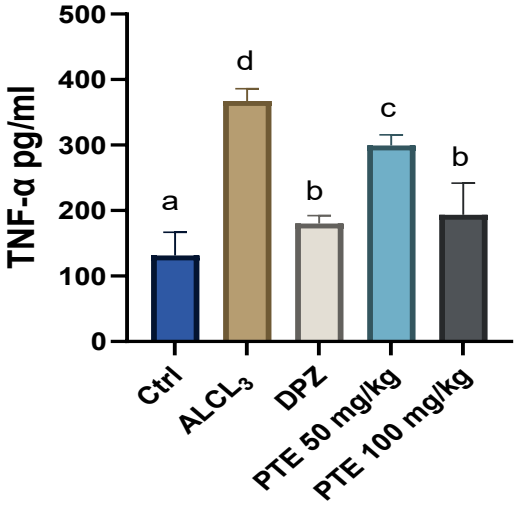

Figure 9: Effect of pterostilbene on tumor necrosis factor alpha (TNF-α) among different groups: Results are expressed by bars representing means± standard deviation (SD). The statistical analysis was done using one-way ANOVA followed by Tukey's multiple comparisons test (n=8): CTRL=control, $AlCl_3$=aluminum chloride (induction), DPZ=donepezil(standard), PTE=pterostilbene. Different lowercase letters indicate a statistically significant difference between groups (p < 0.05).

Histopathological examination

Pathological changes in the cerebral cortex were seen in all groups, as illustrated in **(Fig.10)**. The portions were evaluated visually according to the scoring method indicated in **(Table 1)**. The induction group (mean rank = 20) demonstrated a substantial elevation in Secondary amyloidosis relative to the control group (mean rank = 10). Furthermore, dosages of 50 mg/kg and 100 mg/kg of pterostilbene produced a modest, almost non-significant reduction (P > 0.05) relative to the induction group, with mean rankings of 12.4 for each. Administration of 1 mg/kg of donepezil demonstrates a significant reduction, with mean ranks of 10, in comparison to the induction group.

Table 1: Histopathologic score system of the studied groups.

Group	Control	Induction	DPZ	PTE 50 mg/kg	PTE 100 mg/kg
Mean Rank	10^{a}	20^{b}	10^{a}	12.4^{ab}	12.4^{ab}
Score Value	0	1	0	0	0

- Data are presented as mean ranks (non-parametric, Kruskal-Wallis one-way ANOVA followed by Dunn's post hoc test was used to identify pairwise group differences); differing lowercase letters indicate significant differences ($P \leq 0.05$) across groups.
- Intensity of cellular alterations: 0 (no change), 1 (Weak), 2 (Moderate), 3 (Severe).
- DPZ =Donepezil, PTE =Pterostilbene

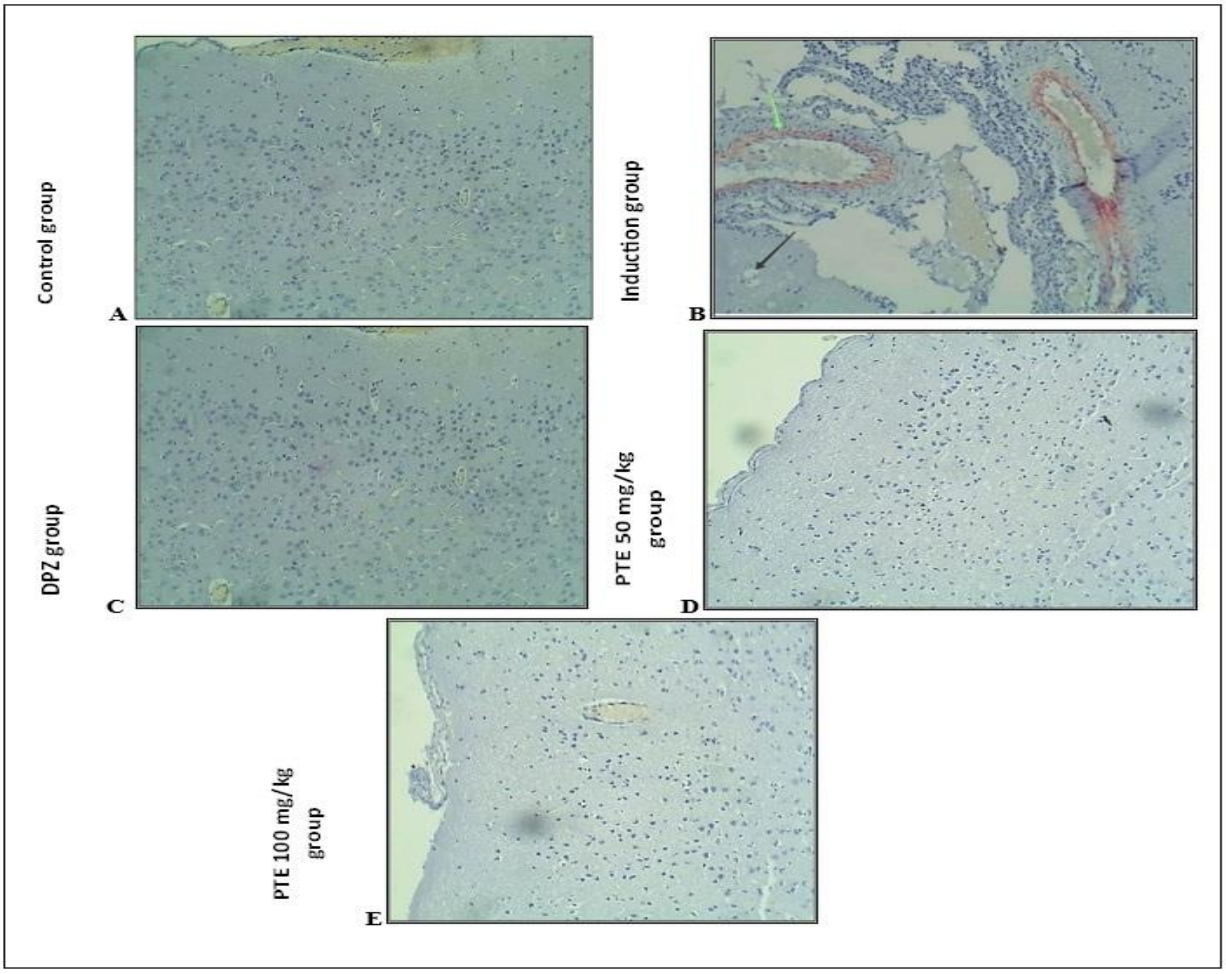

FIGURE 10 |Representative histological images of the cerebral cortex section. A. The control group shows an apparently normal appearance and arrangement of the neurons. B. The induction group shows marked vascular amyloidosis in meningeal arteries (green arrows), venous congestion & vascular degeneration of molecular cerebral layers neurons (black arrows). C. The DPZ and D. PTE 50 mg/kg group and E. PTE 100 mg/kg group show an apparently normal appearance (100X, Congo red stain). DPZ = donepezil, PTE =pterostilbene.

DISCUSSION

The cause of Alzheimer's disease is unknown, but risk factors include depression, age, head trauma, oxidative stress, neuroinflammation, and exposure to metal pollutants. Aluminum chloride, a toxic molecule, can cause oxidative damage to cellular markers, exacerbate brain injury, and increase amyloid beta accumulation. Studies have shown that AlCl3 toxicity can be used as an experimental model for AD, and various medications have been shown to reduce neurotoxicity caused by AlCl3 exposure (29,30).

Available treatments mostly give symptomatic relief, which is frequently accompanied by negative side effects. The long-term efficacy and safety of these drugs require further proof. Thus, the hunt for safer and more effective Alzheimer's disease treatments continues to be a serious and pressing concern(31).

Researchers have placed a lot of emphasis on the theory that inflammation and oxidative stress play a role in AD development. Numerous studies suggested using natural supplements with antioxidant and anti-inflammatory qualities to regulate oxidative stress and inflammation, which may slow or prevent the onset of AD(29).

Researchers often use the open field test (OFT) to assess the amount and quality of exploratory behavior in rats and mice (32).

Figures (2, 3, 4and 5) illustrate the results. The $AlCl_3$-induced Alzheimer's disease rats demonstrated a reduced number of squares traversed and an increased duration of immobility. These findings align with the previous studies conducted by Elmorsy et al. (2021)(33). However, rearing and fecal pellet counts were not significantly different in induction group compared to the control group, contradicting the study results of Singh et al. (2018), (23).

Moreover, the $AlCl_3$-induced Alzheimer's disease rats received supplements of 50 and 100 mg/kg of PTE, leading to a significant increase in the number of squares crossed and a reduction in the duration of immobility in a dose-dependent manner where the 100 mg/kg of PTE group was the better, while rearing and faecal pellet counts were not significantly different compared to the control group. These findings of the present study are inconsistent with the previous study conducted by Sharma et al. in (2002)(34).

The mechanism of PTE in enhancing locomotion may resemble that of resveratrol (RSV), as demonstrated in the study which evaluated the function of RSV in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) driven rodent model of Parkinson's disease by Guo et al. (2016), who reported that RSV activates SIRT1, resulting in a decrease in the amount of acetylated LC3, the important regulator of autophagy. This leads to the redistribution of LC3 from the nucleus to the cytoplasm, followed by a rise in LC3-II and the autophagic destruction of α-synuclein and p62 in dopaminergic neurons, ultimately enhancing the survival of these neurons and mitigating behavioral impairments. Microtubule-associated protein one light chain 3 (LC3) is a crucial regulator of autophagy, predominantly localized in the nucleus, which induces the autophagic destruction of α-synuclein in dopaminergic neurons (35). For this mechanism, 100 mg/kg of PTE may be better for the treatment of behavioral impairment caused by $AlCl_3$.

Limited research has focused on the levels of 8-epi-prostaglandin F2α (8-epi-PGF2α) in brain homogenate, which is one isomer among up to 64 different isomers of F2-isoprostanes (prostaglandin F2-like compounds). The synthesis of these compounds via the non-cyclooxygenase pathway of prostanoid synthesis involves free radical-catalyzed lipid peroxidation of arachidonic acid, resulting in the formation of

endoperoxides that are subsequently reduced by naturally occurring reducing agents to yield F2-isoprostanes.8-epi-PGF2α has been identified as an indicator of oxidative stress in AD (36,37).

Repeated exposure to $AlCl_3$ increases the level of 8-epi-PGF2α, as seen in **Fig. (6)**. In contrast, administering treatments to the AD animals mitigates this current study agrees with the study by Abdel-Salam et al. (2016) (38).

The 8-iso-prostaglandin F2α and Prostaglandin E2 (PGE2) are byproducts of arachidonic acid; however, 8-epi-PGF2α is produced non-enzymatically by free radicals, while PGE2 is synthesized through a cyclooxygenase (COX)/PGE2 synthase (PGES) pathway. The two serve as markers of oxidative stress(39,40).

The present study aligns with Abd-Elmawla et al. (2023), which demonstrated that pterostilbene and celecoxib greatly mitigated sciatic nerve damage and reduced PGE2 levels following oxaliplatin induction(41).

Free radical-catalyzed lipid peroxidation of arachidonic acid is involved in the production of 8-epi-PGF2α through a prostanoid synthesis route that does not include cyclooxygenase. One possible explanation for how Pterostilbene lowers the 8-epi-PGF2α level is its concentration-dependent antioxidant activity, which targets various free radicals such as DPPH, ABTS, hydroxyl, superoxide, and hydrogen peroxide(42)The data indicate a dose-dependent correlation; the 100 mg/kg PTE group was closest to the control group.

In the presented study, repeated exposure to $AlCl_3$ diminishes the concentration of TAOC, corroborating recent findings by Chen et al. (2021) (29).

As seen in **Fig. (7)**, the administration of PTE to the AD rats alleviates this effect and enhances the TAOC level in a dose-dependent manner. This finding aligns with a recent investigation conducted by Abd-Elmawla et al. (2023) (41).

The mechanism of PTE as an antioxidant may occur via direct and indirect methods. It functions as a reactive oxygen species scavenger, neutralizing deleterious free radicals and averting cellular damage associated with chronic illnesses. Moreover, PTE indirectly boosts the body's antioxidant systems by upregulating enzymes, offering extensive protection against oxidative stress. PTE also demonstrated the activation of AMP-activated protein kinase (AMPK) and AKT phosphorylation, facilitating the translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) from the cytoplasm to the nucleus. This action subsequently amplifies the expression of Nrf2-regulated genes, Quinone Oxidoreductase 1 (NQO1) and Heme Oxygenase-1 (HO-1), highlighting pterostilbene's potent antioxidant properties(43).There is no significant difference between the DPZ and 100 mg/kg PTE groups.

In the presented study, as seen in **Figure 8** $AlCl_3$ increased the level of interleukin-1b (IL-1b), consistent with a prior investigation by Zhang et al. (2018)(44).

Conversely, administering pterostilbene to rats with Alzheimer's disease induced by $AlCl_3$ resulted in a notable decrease in IL-1β levels, as shown in **Figure 8**. Moreover, the data demonstrate a dose-dependent relationship. The findings of the current study correspond with those of Li et al. (2018)(45). This may be the mechanism by which PTE reduces IL-1β levels

Additionally, according to a study by Hou et al. (2015), which examined the neuroprotective properties of natural substances against LPS-induced inflammatory reactions in microglia, it was reported that pterostilbene effectively reduced LPS-induced phosphorylation of MAPKs (p38, ERK, and JNK). This suggests that pterostilbene's ability to disrupt MAPK

signaling pathways may decrease the expression of pro-inflammatory mediators in microglia(46).No significant difference between DPZ and 100 mg/kg PTE group.

The conducted study, as seen in **Figure 9**, demonstrates that $AlCl_3$ elevated the levels of TNF-α, corroborating findings from Cao et al. (2016)(47).

Administration of pterostilbene to rats with Alzheimer's disease induced by $AlCl_3$ significantly reduced TNF-α levels, as seen in **Figure 9.** Furthermore, the findings indicate a dose-dependent correlation. The results of the present investigation align with the previous study of Naik et al. (2017)(48).

Liu et al. (2023) proposed the mechanism that PTE has been found in vitro to reduce the activation of NF-κB, thereby downregulating proinflammatory cytokines like TNF-α and interleukin-1beta (IL-1β) in inflammatory and oncological diseases(43)Moreover, A recent study by Li et al. (2022) demonstrated that PTE mitigated STZ-induced neuroinflammation in the hippocampus by suppressing TNF-α, IL-1β, IL-6, and p-NF-κB levels via the modulation of MAOB(49). There is no significant difference between the DPZ and 100 mg/kg PTE groups.

Subsequently, a histological study of the cortex was conducted. Prolonged treatment of $AlCl_3$ for 30 days revealed that the meningeal arteries within the intracerebral fissure exhibited pronounced vascular amyloidosis, characterized by amyloid accumulation within the subendothelial connective tissue, as shown in the **table (1),** which shows the result of the scoring system. This scoring system provides objective, reproducible, and comparable results across different samples. PTE therapy significantly reversed this pathology, preserving non-pathological brain anatomy as shown in **Fig. 10** and table 1the result of the present study corroborating recent studies by Karuppagounder et al. (2009)(50).

Kumar et al. (2024) stated that, in certain instances, brain scans have shown amyloid plaques in persons without dementia. Conversely, several individuals diagnosed with dementia who received numerous brain scans exhibited no indication of plaques(51). Serrano-Pozo et al. (2011) provided a report. In addition to building up in amyloid plaques in the brain parenchyma, the amyloid peptide can also cause cerebral amyloid angiopathy (CAA) in the walls of blood vessels. The core of senile plaques is occupied by the more insoluble and aggregation-prone Aβ42 peptide. In contrast, the primary component of CAA, the more soluble Aβ40 peptide, mainly builds up in the interstitium between the smooth muscle cells of the tunica media. While CAA can also manifest independently (pure CAA), it is more prevalent when associated with AD. Approximately 80% of AD patients have a modest degree of CAA at autopsy, which aligns with the findings of the present study(52).The plaques progressively accumulated with age in both the lower and upper layers of the brain(53,54)

Zeng et al. (2020) evaluated whether pterostilbene has protective benefits in newborn rats against experimentally induced ischemic brain damage, which is related to Hypoxic-ischemic encephalopathy (HIE). HIE is a major cause of fetal brain injury, leading to death and neurological issues, including cerebral palsy, epilepsy, visual and auditory problems, movement disorders, and cognitive deficits (Grow and Barks 2002; Ferriero 2004). HIE occurs in 1 to 4 per 1000 live births. Who found that pterostilbene reduced neuronal cell death, lessened brain swelling, improved brain structure, and showed antioxidant effects by decreasing reactive oxygen species (ROS) and influencing Nrf2 and HO-1 signaling pathways. Additionally, pterostilbene affected NF-κB signaling and the PI3K/Akt/mTOR-JNK pathway. These findings suggest that pterostilbene is a

promising candidate for further research in treating newborn hypoxic-ischemic brain damage(55).

CONCLUSION

The conducted study showed the anti-AD activity of PTE by reducing oxidative stress and inflammation, and amyloidosis, possibly by decreasing 8-epi-PGF2α, IL-1β and TNF-α and increasing TAOC.

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