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6-Paradol Modulates the Amyloidogenic Pathway and Cognitive Decline in a Streptozotocin Mouse Model of Sporadic Alzheimer's Disease; In-vivo and In-silico Studies

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Abstract

There is a great interest in developing new drugs for the prophylaxis and treatment of Alzheimer's disease (AD) from natural and synthetic sources. 6- Paradol is a principal phenolic constituent in plants belonging to the family Zingiberaceae. This compound's anti-inflammatory, anti-apoptotic, and antioxidant properties suggest that it could be used to treat Alzheimer's disease. In this study, 6- paradol was purified from *Aframomum melegueta* seeds and tested for the possible effect on the STZ-induced Alzheimer's disease model in mice at two dose levels (10 and 20 mg/kg). The curative effects of 6- paradol were assessed by measuring the level of amyloid β 42, α, β, and γ -secretases. Furthermore, the enhancement of cognitive abilities in mice following treatment was evaluated using the Y and water maze. Additionally, docking studies were conducted to analyze how effective 6-paradol could be in treating Alzheimer's in humans, by looking at its interaction with human Aβ 42, α, β and γ-secretase active sites. The results demonstrated that 6-paradol improved cognitive and behavioral impairments as well as AD-like pathology in the streptozotocin model in mice. These beneficial effects were accompanied by a reduction in cerebral Aβ 42, β and γ-secretase activities with increasing of α-secretase activity. Moreover, the experiment showed that 6-paradol binds effectively to the examined active sites, implying that further investigation could be conducted to explore its possible benefits for treating Alzheimer's in humans.

Keywords: Alzheimer's Disease; 6-paradol; secretases; β-amyloid; *Aframomum melegueta*; In-silico Studies

1. Introduction

Alzheimer's Disease (AD) can be regarded as the leading cause of dementia [1]. AD is defined as the progressive degeneration of cognitive and motor functions resulting from the loss of neurons and defects in the neurotransmitters in the brain [1]. Inflammation is a normal reaction of the body to any foreign substance and injury[2]. The brain, like all parts of the body can react to the deposition of various abnormal proteins and to the death of the neurons by inducing an immune response resulting in inflammation in the brain [3]. Neuroinflammation does not exhibit the normal hall marks of inflammation (redness, swelling, heat and pain) because there are no pain fibrils in the brain to initiate those responses [3]. Deposition of Aβ and NFTs in the brain serve as a stimulus of an inflammatory response in the brain [4]. This response is primarily through the activation of microglia and astrocytes. These microglia and astrocytes produce many chemokines and cytokines such as TNF-α, IL-1, IL-6 and many others [5]. Also, microglia and astrocytes induce the complement system activation which leads to the complement-mediated break down of parts of the neurons that are affected by Aβ or NFTs [5]. Reactive oxygen species (ROS) can result from the activation of inflammatory cells leading to neuronal death [6]. Though neuroinflammation is a reaction to certain pathological changes of AD, it exacerbates the progression of the disease when it causes death of neurons that further stimulate the inflammatory response. Astrocytes when activated, produce inflammatory cytokines as microglia and also lead to the over expression of COX-2 which catalyzes the

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conversion of arachidonic acid to several eicosanoids including prostaglandins [5]. These prostaglandins promote the inflammatory response which eventually leads to the production of reactive oxygen species, leading to the death of the neurons and the further progression of Alzheimer's disease [6].

There is a growing interest in the discovery and development of remedies of AD based on several targets such as; choline esterase inhibitors, anti-inflammatory drugs, and secretase inhibitors etc...[7].

Currently, there is only five approved drugs by FDA for the alleviation of AD symptoms, four are (acetyl choline esterase inhibitors) AChEIs i.e. Donepezil, galantamine, rivastigmine, and tacrine and one NMDA antagonist (memantine). Two of the five approved drugs are based on natural product nucleuses namely galantamine an alkaloid of the Amarylliaceae family and rivastigmine a synthetic analogue of physostigmine from Calabar beans [8].

Natural products have long been recognized as a prolific source of bioactive molecules and potential drug candidates [9]. While most of the compounds with anti-Alzheimer effects are plant-derived, only a few are sourced from marine and microbial origins [8]. Among the plant-derived compounds, acetylcholine esterase inhibitors (AChEIs) are the most commonly reported, although antioxidant and anti-inflammatory agents have also shown potential for treating Alzheimer's disease. The alkaloids galantamine[32], physostigmine and its synthetic analogue revastigmine are the most potent AChEI from natural source [8]. The quinolizidine alkaloid, Huperzine A, a natural constituent from the Chinese medicine *Huperzia serrata*, is reported also to be a potent AChEI [10].

Curcumin a major diarylheptanoid from the spice *Curcuma longa* which is commonly used in Asian cuisines has recently attracted interest in the field of treatment of AD [11]. Curcumin inhibited Aβ aggregation and its induced inflammation in *in-vitro* studies. In addition it has inhibitory effect on β-secretase and AChE [11]. Resveratrol, another well-known natural ingredient from grape seeds, is reported to possess a promising curing effect on AD. The mechanism of action of this compound includes anti-oxidant effect and degradation Aβ aggregates [12].

Other plants and plant-constituents with reported anti-Alzheimer effect include bryostatin-1 from *Bugula neritiana*, *Ginko biloba* extract, *Panax ginseng*, *Withania somnifera*, Alpha lipoic acid (ALA) *Opuntia Ficus*, *Punica granatum* and omega-3 fatty acids [13-17].

6-Paradol is a major phenolic constituent in different plants belonging to Zingiberaceae family, that belongs to the hydroxyphenyl alkanes chemical class. It is commonly found in ginger, grain of paradise and cardamomum, which are commonly used in Mediterranean and Asian cuisine [18]. 6-Paradol is structurally similar to gingerol but has one less hydroxyl group in the alkyl chain. While it has been reported as chemopreventive, antioxidant, and anti-inflammatory agent [19] its biological activity is less explored compared to that of gingerol. However, the antioxidant and anti-inflammatory activities of 6-paradol, in addition to the memory-enhancing effects of plants containing it, such as ginger, make it a promising candidate for investigating its effect on AD.

The objective of the current study is to investigate the anti-Alzheimer effect of 6-paradol on biomarkers of Alzheimer's disease and cognitive skills. The potential of widely used plant components, like 6-paradol, as anti-AD agents could impact their use or that of their plant sources in clinical nutrition regimes or as adjuvant therapies for AD.

2. Material and Methods

2.1. Chemicals

6-Paradol was purified from grain of paradise seeds (*Aframomum melgueta*) according to our previous report [18]. The structure of the compound was confirmed by proton and carbon NMR data and by comparison to literature.

2.2. Experimental design

Sporadic Alzheimer's disease (SAD) was induced as previously described [20-22] . Briefly, SAD was induced by intracerebroventricular (ICV) brain injections of 10 µl STZ in a single injection in thiopental anesthetized mice [23].

Animals were divided into five groups (n=10); normal group (GP-1) received only ICV and i.p of 0.9% saline; negative control group (GP-2) received STZ by ICV in a dose 3 mg/kg [24]; (GP-3 and 4) received single dose of STZ (ICV, 3 mg/kg) followed by i.p injection of 6-paradol for 21 consecutive days at dose 10 and 20 mg/kg/day, i.p respectively [25]. Within 24 hours of the last injection, neurobehavioral tests were conducted. All testing was conducted

under top illumination to minimize possible circadian variability.

2.3. Behavioral assessments

2.3.1. Morris Water Maze (MWM)

A circular stainless pool (150 cm (diameter) X 60 cm) was used [26]. Pool was divided into four equal parts and two perpendicular threads were attached to its rims. The pooled was filled with water to its half. A platform was painted black (10 cm X 28 cm), and was placed inside the target quadrant, at 2 cm distance under the water surface. The platform was made invisible by adding purple-colored non-toxic dye to the water to make it opaque. The aim of the task was to enable the mice to learn to swim directly to the platform and reach it in short time under normal conditions. The treatment was performed for five consecutive days and during the first four days, each mouse was subjected to two trials, 15 minutes apart, and the time for each trial should not exceed 2 min. Mouse that succeeded to find the hidden platform in designed time, was kept on the platform for 20 s, before removing it. In case of failure, it was guided to the platform and kept there for 20 s. During the four days, the mean escape latency (MEL), which is the time required for each mouse to find the hidden platform was recorded and used as a measure of learning [27] . On the last day (probe trial), the mice were tested in a trial in which the platform was removed from the pool. Each mouse was allowed one min to explore the water, and the time required to reach the hidden platform location was recorded to assess its ability to memorize the task [27,28].

2.3.2. Y-maze test

The test apparatus is shaped like the letter "Y," with three arms radiating from a central point at equal angles. The arms are typically labeled as the start arm, the novel arm, and the other arm. During the test, the mice is placed at the end of the start arm and allowed to explore the maze freely. The sequence of arm entries and the time spent in each arm are recorded. The test typically consists of two phases: the acquisition phase and the retention phase.

In the acquisition phase, all arms are accessible, and the mice explores the maze during 15 min to familiarize itself with the environment. After intervals of 4 hours, the retention phase begins. One of the arms, either the novel arm or the other arm, is blocked, preventing access to that arm. The mice are then placed back in the start arm and allowed to explore the remaining two arms. The test measures the mice's preference for novelty and its ability to remember which arm was previously accessible. The behaviour of the mice, such as the number of entries into each arm and the time spent in each arm, is analysed to assess spatial recognition, and working memory. Because mice have an innate desire to explore novelty, mice normally preferred to enter the Novel arm rather than other arm. This behaviour means that, memory and novelty-seeking skills is intact. In case mouse's front paws crossed into an arm a score was reported [29].

2.3.3. Tissue sampling

To measure different biochemical parameters, mice brain was obtained from mice after termination of behavioral tests by decapitation. Brain was washed with salted ice, homogenized to get 10 % homogenate. The supernatants were utilized to estimate brain Aβ1-42 content, α-, β- and γ-secretases activities.

2.3.4. Estimation of biochemical parameters

Estimation of amyloid beta 42 (Aβ42) in brain homogenate was assessed using mouse Aβ42 ELISA kit (Anaspec, Germany) according to manufacturer's instructions [30]. Meanwhile gamma secretase subunit APH-1a was measured using mouse gamma-secretase subunit APH-1A-APH-1a ELISA kit (Catalog No: E0406m, EIAab, China). α-secretase was also assessed using Sensolyte 520 TACE (α-secretase) activity kit (Anaspec, CA, USA), according to the manufacturer's instructions. Finally, β-Secretase was determined fluorometrically using β-Secretase Activity Assay Kit (Abcam, Cambridge, UK)

2.3.5. Statistical analysis

Results are presented as means ± SD. For parametric data, statistical analysis was done using One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. In addition, repeated measure two-way ANOVA followed by Tukey's post-hoc test was used to assess the escape latency in Morris water maze for both treatment and time factors. Brown–Forsythe test and Kolmogorov-Smirnov test were used to test data homogeneity and normality respectively. GraphPad Prism® software (version 8.0, CA, USA) was used to perform all statistical analysis and $p < 0.05$ was set as the level of significance for all tests.

2.4. Molecular Modeling

Computer-aided docking experiments were carried out using AutoDock Vina [31]. To study the expected effect of 6-paradol on humans in treating Alzheimer's disease; four ligands were selected: Human β-Secretase 1 (BACE1) crystal structures (PDB code : 1FKN) [32], α –secretase (PDB code : 1BJB)[33], γ-Secretase (PDB code : 6IYC) [34], and β-amyloid (PDB code: 1Z0Q)[35]. The ligands were retrieved from the RCSB Protein Data Bank website (https://www.rcsb.org/)[36]. The structures of 6-paradol was drawn using ChemOffice tool (ChemDraw 16.0) assigned with proper 2D orientation [37]. Its energy was minimized using ChemBio3D and was then used as input for AutoDock Vina, in order to carry out the docking simulation. For each protein structure, the binding sites were predicted using the binding site finder of MOE tool and CASTp analysis [38]. The protein preparation was done using the reported standard protocol by removing the co-crystallized ligand, water molecules, and cofactors; the target protein file was prepared by leaving the associated residue with protein using Auto preparation of target protein file AutoDock 4.2 (MGLTools 1.5.6). The graphical user interface program was used to set the grid box for docking simulations. The grid was set so that it surrounds the region of interest in the macromolecule. The docking algorithm provided with AutoDock Vina v.1.2.0 was used to search for the best docked conformation between ligand and protein. During the docking process, a maximum of nine conformers were considered for each ligand. Then, the resulting docking poses were visually examined with BIOVIA Discovery Studio, and interactions with binding pocket residues were studied. Poses fitting into the binding pocket with the top scores and showing useful ligand enzyme contacts were selected [39].

3. Results

3.1. Identification of 6-paradol

The isolated compound was identified using ^{1}H and ^{13}C NMR analysis and by comparing its data to that of the reported literature (Figure 1).

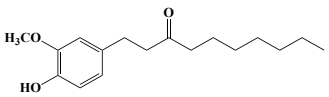

Fig. 1. Chemical structure of 6-paradol

3.2. Effect of 6-Paradol on STZ-induced behavior changes in Morris water maze test

Mice were screened for spatial learning progression as displayed by escape latency in the acquisition phase. STZ-treated mice exhibited an obvious increase in escape latency starting from the 2nd day of the acquisition phase compared to control mice. On the other hand, 6-Paradol treated animals (10 & 20mg/kg) decreased the time required to find the platform compared to the insult (Figure 2A). In the probe test, STZ-treated animals failed to memorize the exact location of the platform as demonstrated by spending less time in the target quadrant to reach 45%, as compared to control group. In contrast, 6-Paradol treated animals 10 & 20mg/kg increased time spent in the target quadrant by 63 and 76%, respectively as compared to control mice [F (3, 36) = 48.95, p < 0.0001] (Figure 2B).

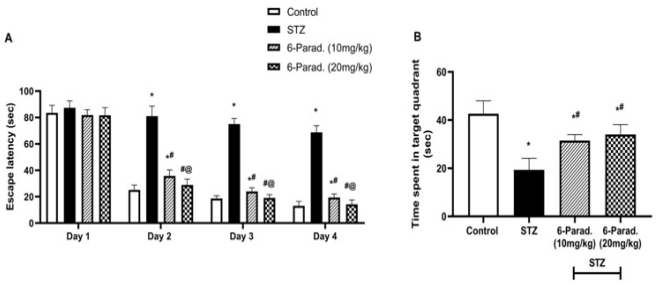

Fig. 2. Effect of 6-Paradol on STZ-induced behavior changes in escape latency [A] and time spent in target quadrant [B] in Morris water maze test. All data were presented as mean ± SD (n = 10), using repeated measure two-way ANOVA followed by Tukey's post-hoc test for escape latency and one-way ANOVA followed by Tukey's post hoc test for time spent in target quadrant; $p < 0.05$. * vs control group, # vs STZ group, @ vs STZ+6-Paradol (10 mg/kg).

3.3. Effect of 6-Paradol on STZ-induced behavior changes in Y-maze test

Y-maze is used to evaluate short-term memory in mice that is assessed by inserting the tested mouse into the Y-maze with one arm blocked during the training session, this arm is designed as novel arm. After 4hr- interval, the mouse is returned to the maze with blockade removal (Testing session). Mouse that recall the previously visited arms and show less tendency to enter the visited arm is a proved to have intact working memory, and consequently intact prefrontal cortical functions. Indeed, STZ-treated mice showed an impairment in short-term memory that evidenced by decreased novel arm count (30%), as compared to normal control. In opposition, 6-

Paradol treated mice (10 & 20mg/kg) reversed the aforementioned parameter and increased the novel arm count by 1 and 2.1-folds, as compared to STZ-treated mice. Moreover, the result of high dose of 6-Paradol (20mg/kg) is statistically significant from the low dose (10 mg/kg) [F (3, 36) = 60.31, p < 0.0001; Figure 3].

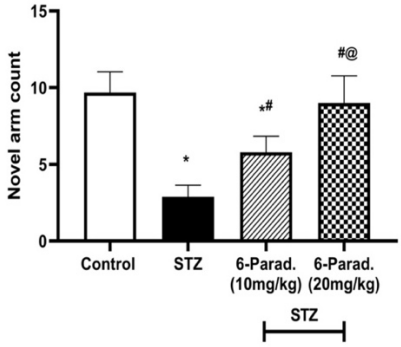

Fig. 3. Effect of 6-Paradol on STZ-induced behavior changes in Y-maze test. All data were presented as mean ± SD (n = 10), using one-way ANOVA followed by Tukey's post hoc test; p < 0.05. * vs control group, # vs STZ group, @ vs STZ+6-Paradol (10 mg/kg).

3.4. Effect of 6-Paradol on STZ-induced alteration in amyloid β-42 level

As depicted in Figure 4, STZ-injected mice showed massive increase in amyloid β (Aβ)-42 level by 1.9-folds, as compared to control group. On the contrary, treatment with 6-Paradol (10 & 20mg/kg) decreased Aβ-42 level by 44 and 64%, as compared to the insult group. Moreover, the high dose of 6-Paradol (20mg/kg) displayed marked reduction in Aβ-42 compared to low dose (10 mg/kg) [F (3, 20) = 113.3, p < 0.0001].

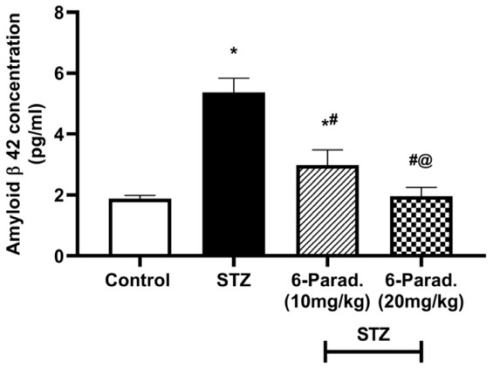

Fig. 4. Effect of 6-Paradol on STZ- induced alteration in amyloid β-42 level. All data were presented as mean ± SD (n = 6), using one-way ANOVA followed by Tukey's post hoc test; p < 0.05. * vs control group, # vs STZ group, @ vs STZ+6-Paradol (10 mg/kg).

3.5. Effect of 6-Paradol on STZ-induced alteration in β and γ secretase activities

One of the pathological features of AD is senile plaques that contain amyloid β-peptide (Aβ). The latter is produced by proteolytic cleavage of amyloid precursor protein (APP) by the action of β and γ secretase. Indeed, ICV injection of STZ demonstrated an increase in β secretase activity and γ secretase level by 1.7 and 1.6-folds, as compared to the control group. On the other hand, 6-Paradol treated mice (10 & 20mg/kg) displayed a marked decline in β-secretase activity by 43 and 52% [F (3, 20) = 973.1, p < 0.0001] as well as γ-secretase level by 42 and 60% [F (3, 20) = 117.1, p < 0.0001], respectively as compared to STZ-injected mice (Figure 5). Additionally, the result of high dose of 6-Paradol (20mg/kg) is statistically significant from the low dose (10 mg/kg).

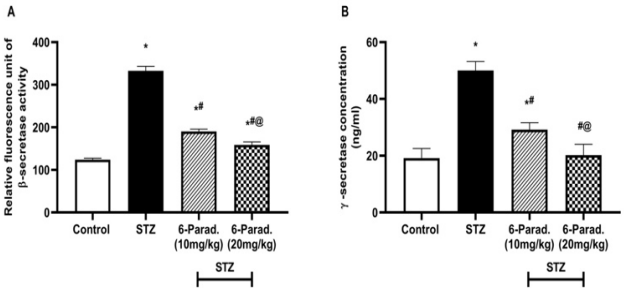

Fig. 5. Effect of 6-Paradol on STZ-induced alteration in β secretase activity [A] and γ secretase level [B]. All data were presented as mean ± SD (n = 6), using one-way ANOVA followed by Tukey's post hoc test; p < 0.05. * vs control group, # vs STZ group, @ vs STZ+6-Paradol (10 mg/kg).

3.6. Effect of 6-Paradol on STZ-induced alteration in α-secretase activity

In contrast to β- and γ-secretase; α-secretase cleaves APP within the Aβ domain, thus preventing Aβ generation. In addition, it yields a secreted APP ectodomain that has neurotrophic and neuroprotective properties [40]. Therefore, α-secretase is pretended to play a chief role in the prevention of the molecular mechanisms underlying AD. In the current study, STZ-injected mice showed an obvious reduction in α-secretase activity by 70%, as compared to control group. Contrariwise, treatment with 6-Paradol (10 & 20mg/kg) increased α-secretase activity to reach 1.5 and 2.2-folds, respectively as compared to the AD model [F (3, 20) = 100.3, p < 0.0001; Figure 5]. Moreover, the high dose of 6-Paradol (20mg/kg) revealed prominent upsurge in α-secretase activity compared to low dose (10 mg/kg).

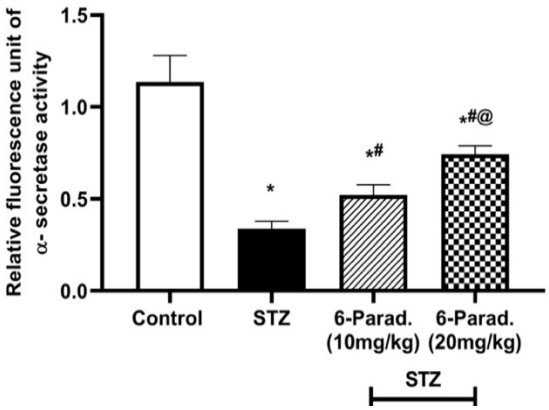

Fig. 6. Effect of 6-Paradol on STZ-induced alteration in α-secretase activity**.** All data were presented as mean ± SD (n = 6), using one-way ANOVA followed by Tukey's post hoc test; $p < 0.05$. * vs control group, # vs STZ group, @ vs STZ+6-Paradol (10 mg/kg).

3.7. Molecular Docking Study

To learn more about the molecular interactions between 6-Paradol and the target receptors, molecular docking was carried out. There is no co-crystallized ligand for Human β-Secretase 1 (BACE1) crystal structures (PDB code : 1FKN), so the MOE site finder module was utilized for active pocket prediction. The docked 6-Paradol Human - β-Secretase complex has established a reliable hydrogen bonding interaction with Lys 224 and Thr 329. Additionally, a Pi-alkyl interaction between Leu 30, Tyr 71, and the aliphatic side chain with a docking score of -6.38 K.cal/mol was detected. Figure 7.

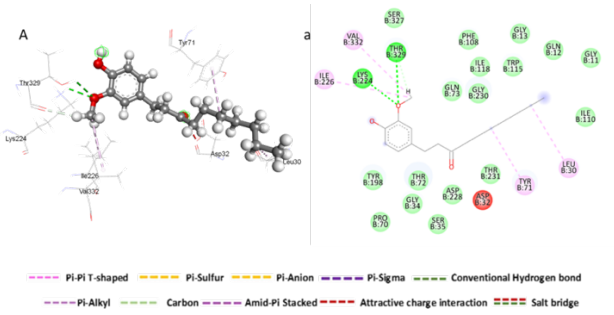

Fig. 7. Molecular visualization of 6-Paradol at the catalytic binding sites of Human β-Secretase 1 (BACE1) receptor.

Furthermore, a secure hydrogen interaction between 6-Paradol and His 6 was showed in the docked Human α -secretase -6-Paradol complex. The Pi-Alkyl interaction between His 13, Leu 17, and the aliphatic side chain, with a docking score of -6.72 Kcal/mol. Figure 8.

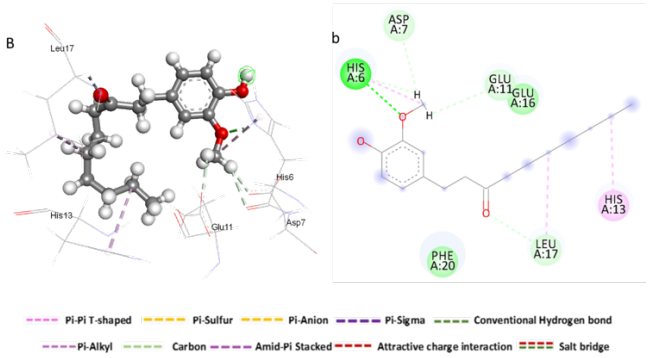

Fig. 8. Molecular visualization of 6-Paradol at the catalytic binding sites of Human α –secretase receptor.

In addition, the docked Human γ -secretase -6-Paradol complex demonstrated that Thr 687 and 6-Paradol had established a secured hydrogen bond. A pharmacophoric hot spot Phe 698 residue has also created a Pi-Pi interaction with an aliphatic side chain and a methoxy group. Pi-alkyl contact between Arg115, Val176, Ala232, and the aliphatic side chain has finally been created, with a docking score of -5.04 Kcal/mol. Figure 9.

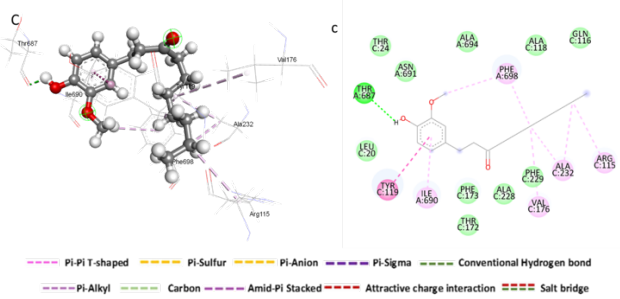

Fig. 9. Molecular visualization of 6-Paradol at the catalytic binding sites of γ-Secretase receptor

Finally, 6-Pardol has developed Pi-alkyl contact between the aliphatic side chain and Ile 31, Ile 34 residues, as seen by the docked Human β-amyloid complex. A pharmacophoric hot spot Val 39 residue has also established Pi-alkyl interaction with methoxy group and phenyl ring. Figure 10.

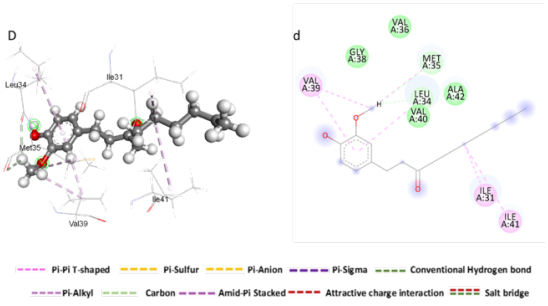

Fig. 10. Molecular visualization of 6-Paradol at the catalytic binding sites of β-amyloid receptor

4. Discussion

The major clinical characteristic of AD patients is the progressive decline in cognition [1]. Central administration of STZ (streptozotocin) has been proven to deteriorate memory in a similar manner to Alzheimer's [41]. Cognitive dysfunction is thought to be caused by the up-regulation of pro-inflammatory cytokines [3]. There is a well-established link between STZ-caused cognitive impairment and inflammatory cytokines expression [41]. Researchers have demonstrated that correcting an excessive inflammatory response improves cognitive function. Hence, the STZ mouse model is an effective model to evaluate cognitive impairment [41].

In studies of memory and spatial learning, Morris water mazes are widely used. In the current study, the effect of STZ, and 6-paradol on cognitive dysfunction in rats was tested using the Morris water maze. The obtained results proved that STZ significantly increases escape latency and decreases the time spent in the fourth quadrant. Moreover, the study concluded that mice treated with ICV-STZ had impairments in spatial learning and memory based on impaired acquisition and retention in passive avoidance and MWM [42].

Additionally, mice failed to recall the platform location during the probe trial on the last day, and spending less time in the target quadrant compared to the normal group [43]. In the modified ç test, STZ also showed significant impairments in novelty-seeking behaviors and spatial memory as proved previously [44].

Currently, small molecular compounds are being investigated for their potential to modulate Alzheimer's disease. Accordingly, certain dietary-derived substances from herbal compounds were examined for their potential anti-degenerative and anti-aging properties [45]. Recently, researchers have focused attention on phenolic compounds, among them flavonoids, which might be anti-amyloidogenic [46]. Recent findings suggest that pretreatment with hesperidin improves cognitive impairment induced by STZ-ICV injection coincides with the beneficial behavioral neuroprotective effects of the studied phenolic; 6-paradol [47]. It was proved that Cyclooxygenase-2 appears to be involved in these effects through its inhibition of inflammation markers [47].

An earlier study demonstrated that [6]-paradol, [6]-gingerol, and [6]-shogaol isolated from grains of paradise (*A. melegueta*) showed promising anti-inflammatory effects through inhibition of COX-2 enzyme activity and gene expression [48].

In the current study, treatment with 6-paradol ameliorate the elevated levels of Aβ-42, β and γ-secretases and increase in the activity of α-secretase. There has been recent research indicating that polyphenols from tea, citrus bioflavonoids, grape-derived polyphenols, and caffeine have anti-amyloidogenic effects [46].

It was reported that α- and β-secretases compete for β-Amyloid precursor protein (APP) proteolysis [49]. Therefore, in this study, cerebral Aβ pathology and weakened Aβ secretion is related to reduced β-secretase activity. A similar effect was demonstrated in an earlier study using the Tg2576 mouse model for cerebral amyloidosis where EGCG, was also found to promote APP metabolism in a non-amyloidogenic manner. EGCG promotes the expression of α-secretase, a disintegrin/metalloprotease, which may be the main mechanism by which EGCG affect nonamyloidogenic APP processing [50]. Moreover, it was observed that the polyphenol tannic acid (TA), has been found to reverse behavioral impairment and AD-like pathology in transgenic mice through decreasing of neuroinflammation and cerebral Aβ levels [51].

Finally, an *in vivo* neuroinflammatory model involving lipopolysaccharide (LPS) was used to examine the effect of shogaol, a pungent agent from *Zingiber officinale* related to the studied compound; 6-paradol. Shogaol, inhibits prostaglandin E(2) and downregulates COX-2 expression more effectively than 6-gingerol, thus exerting anti-inflammatory effects [52].

Collectively, the well-known pathological features of Alzheimer's disease, including the existence of amyloid-beta plaques, tau protein tangles, inflammation [41], oxidative stress [53], and

excitotoxic cell death [54], may be counteracted by the anti-inflammatory, anti-apoptotic, and antioxidant properties exhibited by 6-paradol [55].

In this study 6-Paradol showed promising activity against Alzheimer's disease through reduction in cerebral Aβ 42, β and γ-secretase activities with increasing of α-secretase activity. However, it's important to note that the results obtained from studies in mice may not always directly translate to humans. Therefore, the authors were intrigued to forecast the impact of 6-paradol on humans in treating Alzheimer's and had to anticipate its action on amyloid and secretases. Docking studies involve computational simulations to predict the binding interactions between a ligand (in this case, 6-Paradol) and a target protein (such as amyloid-β and secretase receptors). Our research indicated that 6-paradol binds effectively to the receptors we studied, suggesting that further research could be conducted to uncover its potential for treating Alzheimer's in humans.

Docking experiments can offer clues regarding the possible binding strength and interactions of a compound, yet they cannot be relied upon as the ultimate decision-maker for determining its potency and impact on people. When it comes to Alzheimer's disease, it is difficult to form successful treatments. Numerous approaches that have been encouraging in earlier experiments have not been able to deliver the same results in clinical trials conducted on humans. The complexity of the illness and the disparities between animals used for research and actual human biology complicate the situation.Therefore, Further studies, would be necessary to determine its efficacy and safety profile as a potential treatment for Alzheimer's disease.

5. Conclusions

Based on our findings, we conclude that 6-paradol, a plant-derived phenolic, can improve cognitive and behavioural impairment and AD-like pathology in a streptozotocin mouse model. This effect is likely due to the reduction of Aβ 42, β-and γ secretase activities. 6-paradol has been found to be an effective prophylactic for AD because of its ability to reduce the levels of Aβ, which is a major contributor to the progression of the disease. Furthermore, the fact that 6-paradol is a naturally occurring compound found in many plants means that it can be taken as a supplement without the need for expensive pharmaceutical drugs. Moreover, our findings showed that in docking studies 6-paradol binds well to the human receptors we evaluated, implying that additional exploration should be done to explore its possibility as a treatment for Alzheimer's in humans. All in all, our research suggests that 6-paradol could be a promising alternative to traditional treatments for Alzheimer's disease, and further research is necessary to fully understand its potential.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

1. Malik, R.; Kalra, S.; Bhatia, S.; Al Harrasi, A.; Singh, G.; Mohan, S.; Makeen, H.A.; Albratty, M.; Meraya, A.; Bahar, B. Overview of therapeutic targets in management of dementia. *Biomed. pharmacother.* **2022**, 113168.
2. Ji, J.; Yuan, M.; Ji, R.-R. Inflammation and Pain. In *Neuroimmune Interactions in Pain: Mechanisms and Therapeutics*, Springer: 2023; pp. 17-41.
3. Thakur, S.; Dhapola, R.; Sarma, P.; Medhi, B.; Reddy, D.H. Neuroinflammation in Alzheimer's disease: current progress in molecular signaling and therapeutics. *Inflammation* **2023**, *46*, 1-17.
4. Islam, R.; Choudhary, H.; Rajan, R.; Vrionis, F.; Hanafy, K.A. An overview on microglial origin, distribution, and phenotype in Alzheimer's disease. *J. Cell. Physiol.* **2022**, 1-15.
5. Singh, D. Astrocytic and microglial cells as the modulators of neuroinflammation in Alzheimer's disease. *J. Neuroinflammation* **2022**, *19*, 206.
6. Beura, S.K.; Dhapola, R.; Panigrahi, A.R.; Yadav, P.; Reddy, D.H.; Singh, S.K. Redefining oxidative stress in Alzheimer's disease: Targeting platelet reactive oxygen species for novel therapeutic options. *Life Sci.* **2022**, 120855.
7. Sahana, S.; Kumar, R.; Nag, S.; Paul, R.; Chatterjee, I.; Guha, N. A Review On Alzheimer Disease And Future Prospects. *World Journal of Pharmacy and Pharmaceutical Sciences* **2020**, *9*, 1276-1285
8. Long, J.M.; Holtzman, D.M. Alzheimer disease: an update on pathobiology and treatment strategies. *Cell* **2019**, *179*, 312-339.
9. Gurnani, N.; Mehta, D.; Gupta, M.; Mehta, B. Natural products: source of potential drugs. *Afr J Basic Appl Sci* **2014**, *6*, 171-186.
10. Yan, Y.-P.; Chen, J.-Y.; Lu, J.-H. Disease-Modifying Activity of Huperzine A on Alzheimer's Disease: Evidence from Preclinical Studies on Rodent Models. *Int. J. Mol. Sci.* **2022**, *23*, 15238.
11. Zhou, X.; Venigalla, M.; Raju, R.; Münch, G. Pharmacological considerations for treating

neuroinflammation with curcumin in Alzheimer's disease. *J. Neural Transm.* **2022**, *129*, 755-771.
12. Tosatti, J.A.G.; Fontes, A.F.d.S.; Caramelli, P.; Gomes, K.B. Effects of resveratrol supplementation on the cognitive function of patients with Alzheimer's disease: a systematic review of randomized controlled trials. *Drugs & Aging* **2022**, *39*, 285-295.
13. Tian, Z.; Lu, X.-T.; Jiang, X.; Tian, J. Bryostatin-1: A promising compound for neurological disorders. *Front. Pharmacol. 14*, 1499.
14. Hassan, N.A.; Alshamari, A.K.; Hassan, A.A.; Elharrif, M.G.; Alhajri, A.M.; Sattam, M.; Khattab, R.R. Advances on Therapeutic Strategies for Alzheimer's Disease: From Medicinal Plant to Nanotechnology. *Molecules* **2022**, *27*, 4839.
15. Murad, S.A.; Abd-Elshafy, D.N.; Abou Baker, D.H.; Bahgat, M.M.; Ibrahim, E.A.; Gaafar, A.A.; Salama, Z. Unveiling The Anti-Alzheimer, Antioxidant, Anti-Inflammatory, Antiviral Therapeutic Functionality Of Polysaccharides Extracted From Opuntia Ficus. *Egyptian Journal of Chemistry* **2023**, *66*, 237-244.
16. Ali, A.A.E.-M.; El-Hallouty, S.M.; El-Desouky, M.A. Amelioration of Alzheimer's disease with extracts of Punica granatum and Persea americana in AlCl3 induced rats. *Egyptian Journal of Chemistry* **2023**, *66*, 21-32.
17. Elhallouty, S.M.; Rashad, A.M.; Abd Elrhman, E.A.; Elkaramany, H.A.K.; Ibrahim, M.G.; Salem, N.T.; Adeeb, R.A.; Ibrahim, Y.W.; El Shahed, Z.A.E. Effect of Ginkgo biloba leaf extract in combination with vitamin C, E and D on Aluminum Chloride induced Alzheimer in rats. *Egyptian Journal of Chemistry* **2022**, *65*, 827-841.
18. Rafeeq, M.; Murad, H.A.S.; Abdallah, H.M.; El-Halawany, A.M. Protective effect of 6-paradol in acetic acid-induced ulcerative colitis in rats. *BMC Complement. Med. Ther.* **2021**, *21*, 1-10.
19. Suresh, K.; Manoharan, S.; Arokia Vijayaanand, M.; Sugunadevi, G. Chemopreventive and antioxidant efficacy of (6)-paradol in 7, 12-dimethylbenz (a) anthracene induced hamster buccal pouch carcinogenesis. *Pharmacol. Rep.* **2010**, *62*, 1178-1185.
20. Pelleymounter, M.A.; Joppa, M.; Carmouche, M.; Cullen, M.J.; Brown, B.; Murphy, B.; Grigoriadis, D.E.; Ling, N.; Foster, A.C. Role of corticotropin-releasing factor (CRF) receptors in the anorexic syndrome induced by CRF. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 799-806.
21. Pelleymounter, M.A.; Joppa, M.; Ling, N.; Foster, A.C. Pharmacological evidence supporting a role for central corticotropin-releasing factor2 receptors in behavioral, but not endocrine, response to environmental stress. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 145-152.
22. Sirwi, A.; El Sayed, N.S.; Abdallah, H.M.; Ibrahim, S.R.; Mohamed, G.A.; El-Halawany, A.M.; Safo, M.K.; Abdel Rasheed, N.O. Umuhengerin Neuroprotective Effects in Streptozotocin-Induced Alzheimer's Disease Mouse Model via Targeting Nrf2 and NF-Kβ Signaling Cascades. *Antioxidants* **2021**, *10*, 2011.
23. Hindam, M.O.; Sayed, R.H.; Skalicka-Woźniak, K.; Budzyńska, B.; El Sayed, N.S. Xanthotoxin and umbelliferone attenuate cognitive dysfunction in a streptozotocin-induced rat model of sporadic Alzheimer's disease: The role of JAK2/STAT3 and Nrf2/HO-1 signalling pathway modulation. *Phytother. Res.* **2020**, *34*, 2351-2365.
24. Deshmukh, R.; Sharma, V.; Mehan, S.; Sharma, N.; Bedi, K. Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine—a PDE1 inhibitor. *Eur. J. Pharmacol.* **2009**, *620*, 49-56.
25. Gaire, B.P.; Kwon, O.W.; Park, S.H.; Chun, K.-H.; Kim, S.Y.; Shin, D.Y.; Choi, J.W. Neuroprotective effect of 6-paradol in focal cerebral ischemia involves the attenuation of neuroinflammatory responses in activated microglia. *PLoS One* **2015**, *10*, e0120203.
26. D'Hooge, R.; De Deyn, P.P. Applications of the Morris water maze in the study of learning and memory. *Brain Res. Rev.* **2001**, *36*, 60-90.
27. Singh, S.; Kaur, H.; Sandhir, R. Fractal dimensions: A new paradigm to assess spatial memory and learning using Morris water maze. *Behav. Brain Res.* **2016**, *299*, 141-146.
28. Blokland, A.; Geraerts, E.; Been, M. A detailed analysis of rats' spatial memory in a probe trial of a Morris task. *Behav. Brain Res.* **2004**, *154*, 71-75.
29. Granon, S.; Save, E.; Buhot, M.-C.; Poucet, B. Effortful information processing in a spontaneous spatial situation by rats with medial prefrontal lesions. *Behav. Brain Res.* **1996**, *78*, 147-154.
30. Masliah, E.; Mallory, M.; Alford, M.; DeTeresa, R.; Hansen, L.; McKeel, D.; Morris, J. Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. *Neurology* **2001**, *56*, 127-129.
31. Trott, O.; Olson, A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455-461.
32. Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A.K.; Zhang, X.C.; Tang, J. Structure of the protease domain of memapsin 2 (β-secretase) complexed with inhibitor. *Science* **2000**, *290*, 150-153.
33. Poulsen, S.-A.; Watson, A.A.; Fairlie, D.P.; Craik, D.J. Solution structures in aqueous SDS micelles of two amyloid β peptides of Aβ (1–28) mutated at the α-secretase cleavage site (K16E, K16F). *J. Struct. Biol.* **2000**, *130*, 142-152.
34. Zhou, R.; Yang, G.; Guo, X.; Zhou, Q.; Lei, J.; Shi, Y. Recognition of the amyloid precursor protein by human γ-secretase. *Science* **2019**, *363*, eaaw0930.
35. Tomaselli, S.; Esposito, V.; Vangone, P.; van Nuland, N.A.; Bonvin, A.M.; Guerrini, R.; Tancredi, T.; Temussi, P.A.; Picone, D. The α-to-β conformational transition of Alzheimer's Aβ-(1–42) peptide in aqueous media is reversible: a step by step conformational analysis suggests the location of β conformation seeding. *ChemBioChem* **2006**, *7*, 257-267.
36. Berman, H.M.; Battistuz, T.; Bhat, T.N.; Bluhm, W.F.; Bourne, P.E.; Burkhardt, K.; Feng, Z.; Gilliland, G.L.; Iype, L.; Jain, S. The protein data bank. *Acta Crystallogr. Sect. D. Biol. Crystallogr.* **2002**, *58*, 899-907.
37. Halford, B. Reflections on CHEMDRAW. *Chem. Eng. News* **2014**, *92*, 26-27.
38. Tian, W.; Chen, C.; Lei, X.; Zhao, J.; Liang, J. CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res.* **2018**, *46*, W363-W367.
39. Zeleke, D.; Eswaramoorthy, R.; Belay, Z.; Melaku, Y. Synthesis and antibacterial, antioxidant, and molecular docking analysis of some novel quinoline derivatives. *J. Chem.* **2020**, *2020*, 1-16.
40. F Lichtenthaler, S. Alpha-secretase cleavage of the amyloid precursor protein: proteolysis regulated by signaling pathways and protein trafficking. *Curr. Alzheimer Res.* **2012**, *9*, 165-177.
41. Salkovic-Petrisic, M.; Knezovic, A.; Hoyer, S.; Riederer, P. What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research. *J. Neural Transm.* **2013**, *120*, 233-252.
42. Meehan, W.P.; Zhang, J.; Mannix, R.; Whalen, M.J. Increasing recovery time between injuries improves cognitive outcome after repetitive mild concussive brain injuries in mice. *Neurosurgery* **2012**, *71*, 885-892.
43. Singh, B.; Sharma, B.; Jaggi, A.S.; Singh, N. Attenuating effect of lisinopril and telmisartan in intracerebroventricular streptozotocin induced experimental dementia of Alzheimer's disease type: possible involvement of PPAR-γ agonistic property. *J. Renin-Angiotensin-Aldosterone Syst.* **2013**, *14*, 124-136.
44. Wright, R.L.; Conrad, C.D. Short CommunicationChronic stress leaves novelty-seeking behavior intact while impairing spatial recognition memory in the Y-maze. *Stress* **2005**, *8*, 151-154.
45. Peng, Y.; Tao, H.; Wang, S.; Xiao, J.; Wang, Y.; Su, H. Dietary intervention with edible medicinal plants and derived products for prevention of Alzheimer's disease: A

compendium of time-tested strategy. *J. Funct. Foods* **2021**, *81*, 104463.
46. Wang, Q.; Dong, X.; Zhang, R.; Zhao, C. Flavonoids with potential anti-amyloidogenic effects as therapeutic drugs for treating Alzheimer's disease. *J. Alzheimer's Dis.* **2021**, *84*, 505-533.
47. Wang, D.; Liu, L.; Zhu, X.; Wu, W.; Wang, Y. Hesperidin alleviates cognitive impairment, mitochondrial dysfunction and oxidative stress in a mouse model of Alzheimer's disease. *Cell. Mol. Neurobiol.* **2014**, *34*, 1209-1221.
48. Ilic, N.M.; Dey, M.; Poulev, A.A.; Logendra, S.; Kuhn, P.E.; Raskin, I. Anti-inflammatory activity of grains of paradise (Aframomum melegueta Schum) extract. *J. Agric. Food. Chem.* **2014**, *62*, 10452-10457.
49. Gandhi, S.; Refolo, L.M.; Sambamurti, K. Amyloid precursor protein compartmentalization restricts β-amyloid production. *J. Mol. Neurosci.* **2004**, *24*, 137-143.
50. Rezai-Zadeh, K.; Shytle, D.; Sun, N.; Mori, T.; Hou, H.; Jeanniton, D.; Ehrhart, J.; Townsend, K.; Zeng, J.; Morgan, D. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J. Neurosci.* **2005**, *25*, 8807-8814.
51. Mori, T.; Rezai-Zadeh, K.; Koyama, N.; Arendash, G.W.; Yamaguchi, H.; Kakuda, N.; Horikoshi-Sakuraba, Y.; Tan, J.; Town, T. Tannic acid is a natural β-secretase inhibitor that prevents cognitive impairment and mitigates Alzheimer-like pathology in transgenic mice. *J. Biol. Chem.* **2012**, *287*, 6912-6927.
52. Ha, S.K.; Moon, E.; Ju, M.S.; Kim, D.H.; Ryu, J.H.; Oh, M.S.; Kim, S.Y. 6-Shogaol, a ginger product, modulates neuroinflammation: A new approach to neuroprotection. *Neuropharmacology* **2012**, *63*, 211-223.
53. Huang, W.J.; Zhang, X.; Chen, W.W. Role of oxidative stress in Alzheimer's disease. *Biomedical reports* **2016**, *4*, 519-522.
54. Sharma, V.K.; Singh, T.G.; Singh, S.; Garg, N.; Dhiman, S. Apoptotic pathways and Alzheimer's disease: probing therapeutic potential. *Neurochem. Res.* **2021**, *46*, 3103-3122.
55. Binmahfouz, L.S.; Almukadi, H.; Alamoudi, A.J.; El-Halawany, A.M.; Abdallah, H.M.; Algandaby, M.M.; Mohamed, G.A.; Ibrahim, S.R.; Alghamdi, F.A.; Al-Shaeri, M. 6-Paradol Alleviates Testosterone-Induced Benign Prostatic Hyperplasia in Rats by Inhibiting AKT/mTOR Axis. *Plants* **2022**, *11*, 2602.