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Amelioration of Alzheimer's disease with extracts of *Punica granatum and Persea Americana* in $AlCl_3$ induced rats

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Abstract

Background: Alzheimer's disease is a neurological disease that progresses over time. It is defined by the presence of both extracellular amyloidbeta (Ab) plaques, which are primarily made up of deposited Ab, and intracellular neurofibrillary tangles, which are made up of hyperphosphorylated and abnormally phosphorylated tau protein.**Aim:**This study aimed to investigate the role of Punica granatum and Persea Americana extracts as an agent to decrease neurodegenerative effects of $AlCl_3$ toxicity in male rats..**Methods:**42 male rats were divided in to seven experimental groups . G1 is the negative control group, and G2 is the positive control group.,G3,G4 and G5 received Alcl3(17 mg/kgb.w.)oral daily for one month and treated with punica granatum extract (40 mg/kg b.w.),persa americana extract (33.3 mg/kg b.w.) for three months and reference drug aricept(0.4 mg/kg b.w.)2 weeks of taking it orally respectively.

Results: The mean level of dopamine in brain tissue in positive control decreased significantly compared to negative control. Brain caspase 3 and DNA fragmentation were significantly elevated. The treatment of *Punica granatum* and persea americana extracts alleviated the adverse effect of AlCl3 in the treated groups and enhanced the dopamine level and declined apoptotic markers.

conclusion :Punica granatum and persea Americana has an impact to minimize neurodegenerative effect of AD.

Keywords: Alzheimer disease (AD), Punica granatum, Persea Americana, dopamine, caspase 3

1-Introduction

In developed countries, due to genetic and environmental causes, neurodegenerative illnesses are becoming more common among persons over the age of 65. Alzheimer's disease (AD) is predicted to affect one out of every 85 people in 2050 [1]. AD is a progressive neurodegenerative disease defined by neuronal loss in the brain, which causes short-term memory loss and cognitive deficits. Short-term memory loss is the first clinical symptom, followed by signs of mental and learning difficulties such as forgetting names and words during speaking, mood swings, inability to calculate, and inability to utilize everyday objects and tools [2].Apoptotic neuronal death, overexpression of highly phosphorylated tau proteins [3], neurofibrillary tangles, amyloid plaques (because of degeneration of neuronal processes, beta-amyloid proteins accumulate), oxidative stress, and cholinergic dysfunction are all pathological markers[4].Amyloid plaques are made up of amyloid-β (Aβ), a cleavage product of the amyloid-β protein precursor (AβPP). AβPP is cleaved by β -secretase (BACE 1), followed by γ -secretase, to produce Aβ [5]. The accumulation of Aβ monomers results in oligomers, fibrils, and insoluble amyloid plaques, which disrupt synaptic and neuronal function. producing intracellular conditions conducive to the production of neurofibrillary tangles, resulting in neuronal death and subsequent impairment of neurotransmitter function [6].The other signature protein aggregate in Alzheimer's disease is intracellular neurofibrillary tangles, which are formed up of hyper- and improperly phosphorylated tau protein[7].

Age, family history, apolipoprotein E4 genotype, diabetes, hypertension, obesity, hypercholesterolemia, traumatic brain injury and low education level are all risk factors for AD [8].Early-onset autosomal-dominant AD is linked to mutations in the presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid precursor protein (APP) genes [9].

The most significant risk factor for Alzheimer's disease is advanced age. Furthermore, the age of 65 is utilized to classify Alzheimer's disease. Patients with early-onset Alzheimer's disease (EOAD) develop symptoms before the age of 65, while those with late-onset

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Alzheimer's disease (LOAD) develop symptoms.LOAD is the most frequent form of Alzheimer's disease, with only 10% of cases identified as EOAD, which occurs between 45 and 60. [10].

Heavy metals in the environment are well-known influences on brain development. Heavy metals have been linked to neurodegenerative illnesses such as Alzheimer's disease (AD) and Parkinson's disease (PD) in numerous studies [11]. Environmental variables such as air pollution, nutrition, metals, infections, and others can cause oxidative stress and inflammation, raising the risk of developing Alzheimer's disease.[12]. Air Pollution National Ambient Air Quality Standards (NAAQSs) have established six air pollutants: ozone (O3), nitrogen oxides (NOx), carbon monoxide (CO), particulate matter (PM), sulfur dioxide (SO2), and lead.Studies on animals and cellular models have indicated that high amounts of air pollution can cause damage to the olfactory mucosa and bulb, as well as the frontal cortical region, which is comparable to what is seen in Alzheimer's disease.There is a relationship between oxidative stress, neuroinflammation, and neurodegeneration in people exposed to air pollution, with hyperphosphorylated tau and Aβ plaques in the frontal cortex.Air pollution can increase in Aβ42 production, accumulation, and cognitive impairment[13]. Diet some vitamins, minerals, and micronutrients contain potent antioxidants and anti-inflammatory and free radical scavenging properties that can protect against oxidative damage, neuroinflammation, and subsequent cognitive impairment. In contrast, high meat consumption is strongly linked to an increased risk of Alzheimer's disease, an excessive intake of saturated fats or a vitamin E deficiency[14].

Aluminum (Al) is a heavy metal that affects various cellular metabolic pathways in the central nervous system (CNS), making it one of the heavy metals involved in the development of neurodegenerative diseases [15]. Aluminum chloride ($AlCl_3$) is a neurotoxin that builds up in the brain and interferes with synaptic, cholinergic, and dopaminergic neurotransmission [16]. There are several diagnostic tests for Alzheimer's disease (AD) based on the measurement of Aβ levels in the cerebrospinal fluid (CSF) and "neurofibrillary tangles," which form years before some dementia symptoms occur.MRI can quantify metabolic anomalies to assess brain shrinkage, while positron emission tomography can be used to evaluate glucose metabolism and Aβ load.Unfortunately, because they are intrusive, time-consuming, and expensive, such diagnostic procedures are limited. In addition, the disruption of cholesterol and lipid metabolism in the brain is linked to the production, deposition, and clearance of Aβ, which leads to neuronal dysfunction.In Alzheimer's disease, norepinephrine (NE) and dopamine pathway-related metabolites were drastically reduced. In conclusion, studies of AD patients' CSF or blood samples reveal that amino-acid metabolism, mitochondrial activity, neurotransmitter metabolism, and lipid biosynthesis are all altered[17]. The discovery of AChE inhibitors was first the focus of therapeutic methods for improving defective cholinergic neurotransmission. However, the following investigations discovered the importance of both AChE and BuChE in the pathogenesis of AD and the therapeutic potential of inhibiting both AChE and BuChE. This research has aided in the introduction of inhibition as a therapeutic technique in treating Alzheimer's disease. AChE and BuChE (cholinesterase) inhibitors (ChE-Is) reduce neurotransmitter breakdown by boosting brain ACh levels and improving inadequate brain cholinergic neurotransmission. When ChE-Is inhibit AChE, BuChE, and other cholinesterases, they are categorized as nonspecific, and when they only inhibit AChE, they are classified as specific. Based on the degree of enzyme inhibition, these medications can be classed as reversible, pseudo-irreversible, or irreversible. For example, donepezil (Aricept) is a reversible AchE inhibitor that works centrally by increasing ACh bioavailability in the synaptic cleft[18].

In the United States, five therapy options for cognitive symptoms of Alzheimer's disease are now approved, the most recent of which (memantine) was approved more than a decade ago [19].The European Union has approved four of the five standard-of-care medicines, including three cholinesterase inhibitors (donepezil, galantamine, and rivastigmine) and one N-methyl-D-aspartate receptor antagonist (memantine) [20-24].In 2014, a fixed-dose combination of donepezil and memantine was approved for the treatment of patients with moderate to severe Alzheimer's disease who were on stable donepezil therapy [25]. The donepezil neuroprotective mechanism works by reducing the damage caused by Aβ. In addition, by inhibiting IL-1β and cyclooxygenase-2 production, donepezil can reduce systemic inflammation in the brain and spleen[26].

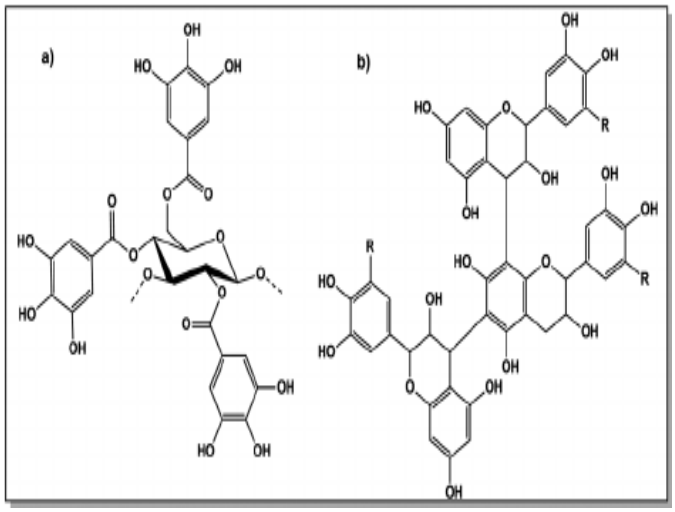
Natural products are gaining popularity as potential medicinal agents. Neuroprotective treatments have shown that animal-derived products like omega-3, fatty acids, and plant-derived substances decrease cellular toxicity and have anti-inflammatory properties [27] Inflammation, which contributes to neurodegeneration, accelerates the progression of Alzheimer's disease. As a result, early prevention and management of inflammation may help treat or lower the symptoms of Alzheimer's disease. Phytochemicals with anti-inflammatory, antioxidant, and neuroprotective characteristics have been shown to have the ability to facilitate and prevent neurodegeneration in Alzheimer's

disease Inflammation, which contributes to neurodegeneration, accelerates the progression of Alzheimer's disease. As a result, early prevention and management of inflammation may help treat or lower the symptoms of Alzheimer's disease. Phytochemicals with anti-inflammatory, antioxidant, and neuroprotective characteristics have been shown to have the ability to facilitate and prevent neurodegeneration in Alzheimer's disease [28].

Punica granatum is a well-known source of nutritionally essential compounds. It includes hydrolyzable tannins, condensed tannins, flavonols, anthocyanins, and phenolic and organic acids components, all of which have been linked to a variety of health benefits [29].Anthocyanins are another important component in the Punica granatum as a functional food. The color of the fruit and its juice are due to these water-soluble plant pigments, members of the flavonoids family fig(1,2). Punica granatum is high in antioxidant polyphenols, which may play a role in neurological disease due to its activity as possible acetylcholinesterase (AChE) inhibitor. Dietary supplementation with 4 percent Punica granatum decreased oxidative damage and reduced AChE activity in AD transgenic mice, restoring normal levels of the enzyme. Additionally, an ethanol extract of Punica granatum leaves or peels inhibited AChE [30]. Antioxidant therapy is one of the treatment options for Alzheimer's disease. Antioxidants help lessen the damage produced by reactive oxygen species and can aid in delaying and avoiding free radical antioxidant responses [31].

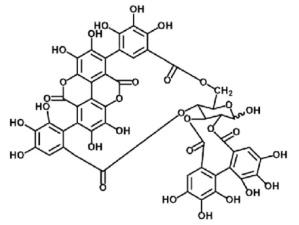
Persea Americana's beneficial components reduce oxidative stress[32] and inflammation [33], control lipids [34], promote cancer cell death [35], induce neuroprotection [36], support memory and brain health [37], and protect against gastric ulcers [38]. Three monounsaturated fatty acids (oleic, palmitoleic, and heptadecenoic acids), two polyunsaturated fatty acids (linolenic and linoleic acids), and seven saturated fatty acids (myristic, palmitic, margaric, stearic, capric, lauric, and pentadecanoic acids) were found in the peels byAna L.Ramos.Aguilar 2021[39]fig(3,4) . Persea Americana is high in phenolic compounds and minerals such as (Ca, Mg, Mn, and Zn). In addition, the antioxidant and AChE inhibitory properties of phenolic compounds were linked [40].

This study aimed to investigate the role of some traditional herbs such as Punica granatum and Persea Americana extracts that may be able to slow the progression of Alzheimer's disease-induced by$AlCl_3$ in experimental animals. In addition, the toxicity or side effect of these extracts, if the present, was also studied.

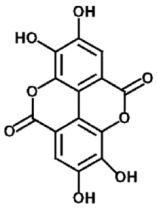


Fig(1-a) hyrolyzable tannins in Punica granatum by-product

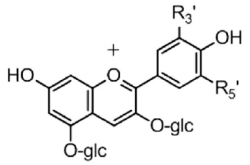
(1-b) condensed tannins



Punicalagin



Ellagic acid



R3'	R5'	Anthocyanin
H	H	Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin

Anthocyanin

Fig(2)

Punica granatum's primary phenolic chemicals have chemical structures. Punicalagin is the most abundant ellagitannins in Punica granatum, while ellagic acid is a tannin representative. The principal polyphenols responsible for Punica granatum's red color are anthocyanins

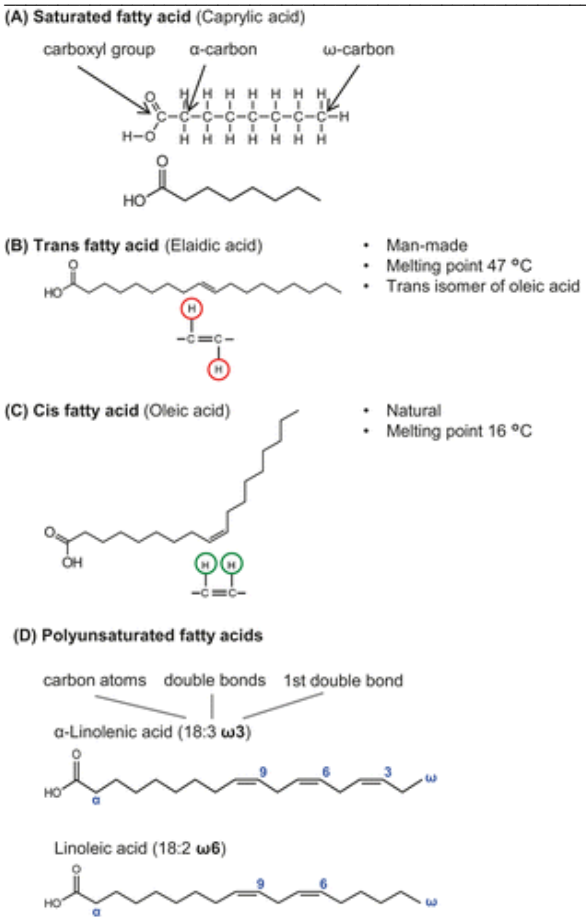
2-Materials and methods

2-1 chemical used

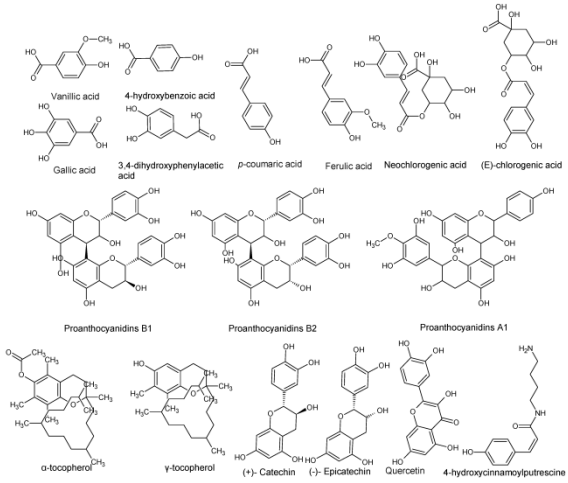
2-1-1 Sigma Chemical Company(USA) provided the aluminium chloride and all other compounds utilised in this study.

2-1-2 Donepezil (purchased from local pharmacy).

2-1-3 Punica granatum and Persea Americana were purchased from a local market



Fig(3) Fatty acids in Persea Americana



Fig(4) Phenolic compounds isolated from Persea Americana.

2-2 Animals and care

A mature male Wister albino rats Rattus norveegicus were used. Their weights ranged between 170-180 gm, at the beginning of the experiment. For one week, they were confined in adequate cages to allow them to adjust to laboratory conditions. Rodent pellets and fresh tap water were always available. These pellets were sourced from the Giza, Egypt-based Agricultural-Industrial Integration Company.

2-3 Plant extracts preparation:

The preparation of methanolic extracts of fruits was carried out using a method previously described by (Basiri et al.,2015)[41] with minor changes.Each fruit was cleaned with tap water, dried in the shade at room temperature, and the peel powdered by a mechanical blender for this purpose.75 g finely crushed peel was then dissolved in 450 ml methanol and stored at room temperature overnight.The extract was filtered through Whatman filter paper no.25 and evaporated at a maximum temperature of 40 ^{o}C in a rotary evaporator.1 gm methanolic extract (P. granatum) was diluted in 100 ml dimethyl sulfoxide (DMSO) to get a final concentration of 40 mg/kg, while 1 gm methanolic extract (P. americana) was dissolved in 120 ml (DMSO) to achieve a final concentration of 33.3 mg/kg[42].

2-4 Induction of Alzheimer's disease(AD) in rats:

The animals were received $AlCl_3$ (17mg/kg body weigh) orally daily for one month. [43].

Diseased animals received Punica granatum (40 mg/Kg b. w.) , Persea Americana extracts (33.3 mg / Kg b. w.) orally on daily for 3 months and reference drug Aricept (0.4 mg/kg b.w) 2 weeks orally and their effects were determined after the administration of the last dose.

2-5 Study design

Animals were divided into seven groups (n = 6) and treated orally as follows:

-Negative control(G1) animals were orally received distilled water .

-Positive control(G2) animals were orally received $AlCl_3$ daily for one month .

-Treated groups animals were orally received two extracts (P.granatum and P. Americana) (G3 and G4) respectively .

-Reference group (G5) animals were orally received Aricept for two weeks.

To study any effect of different extracts (if present) on the experimental animals two groups where studied (G6 and G7) without any exposure to $AlCl_3$.

- Animals were orally received P.granatum extract (G6).
- Animals were orally received P.Americana extract (G7).

2-6 Blood and tissue specimens:

After one month of experiment, the blood samples and brain tissues from positive control (G2) animals were collected. After two weeks of treatment by Aricept blood samples and brain tissues were collected from treated group (G5) . After three months of treatment rats were scarified ,blood samples and brain tissues of treated groups (G3 and G4),negative control(G1) and protected groups (G6 and G7) were collected in clean eppendorff tubes containing disodium EDTA as

anticoagulant for comet assay. Portion of the brain tissues were removed and were frozen for determination of dopamine , caspase 3 and the remaining brains were fixed in 10% buffered-saline formalin for histopathologic evaluation.

Summary diagram of study design:

G1 (negative control) 4 months

G2 (positive control)

One month

Induction AD

G3 (treated with Punica granatum extract)3 months

Induction AD Punica granatum extract

G4 (treated with persea Americana extract) 3 months

Induction AD Persea Americana extract

G5 (treated with reference drug Aricept)

2 weeks

Induction AD Aricept

G6 (negative control + Punica granatum extract) 4 months

Punica granatum extract

G7 (negative control + Persea Americana extract)

4 months

Persea Americana extract

2-7 Biochemical analysis

2-7-1 Brain tissue homogenate preparation:

In a ratio of 1 g/10 ml phosphate buffer, a brain homogenate was produced in ice-cold 50 mM potassium phosphate buffer, pH 7.4, with 1 mM EDTA per gram tissue. The tissue was next homogenised using achilled glass-Teflon Potter-Elvehjem tissue homogenizer. For caspases-3 and dopamine, the homogenate was centrifuged at 10,000 g for 15 minutes at 4 °C, and the supernatant was collected and kept at-20 °C. [44] was used to calculate protein content in brain homogenates.

2-7-2 Determination of dopamine by HPLC

According to[45], brain dopamine was measured using a high-performance liquid chromatography (HPLC) equipment. The mobile phase was a 97/3(v/v) mixture of potassium phosphate buffer and methanol supplied at a flow rate of 1.5ml/min. The injection volume was 20µl and the UV detection was done at 270nm. The peak areas of standards were determined after serial dilutions were injected. By plotting peak areas against corresponding concentrations, a linear standard curve was created. The curve was used to calculate the concentration in the samples.

2-7-3 Determination of caspase 3 using ELISA technique

The manufacturer's instructions(Elabscience Biology Co., Ltd., China) were followed to determine the enzymatic activity of caspase 3 using a rat CASP3. Sandwich-ELISA kit. This kit includes a micro ELISA plate that has been pre-coated with a CASP-3 antibody.

2-7-4 Assay of comet

The comet assay was performed according to [46] with modifications according to [47]. Each damaged cell resembled a comet, with a brightly fluorescent head and a tail to one side generated by DNA strand breaks dragged away during electrophoresis. The percent of damage was calculated by counting the damaged cell out of 100 cells on each slide.

2-7-5 Histopathological examination

Standard techniques were used to fix brain tissue sections in 10% buffered-saline formalin, dehydrate in graded ethanol, and embed in paraffin. Using a light microscope, sections of 4m thickness were stained with hematoxylin and eosin (H&E) for histological investigation [48].

2-8 statistical analysis

Continuous variable data articulated as mean± standard deviation (M± SD) of six animals, and standard computer application (SPSS for Windows,release 25,IBMInc,USA) utilised for data entry and analysis. The variation between groups was assessed using one-way ANOVA, Turkey's honestly significant difference (HSD) test, and statistically significant differences were defined as P-values<0.05.

3-Result

statistical analysis using ANOVA test revealed significant decreased mean level of dopamine in brain tissue in positive control compared to negative control and significant increased mean level of dopamine in brain tissue in treated groups compared to positive group .

Significant increased mean level of caspase 3 in brain tissue in positive control compared to negative control and significant decreased mean level of caspase 3 in brain tissue in treated groups compared to positive group was reported as shown in table (1).

Significant increased mean level of comet in whole blood in positive control compared to negative control and significant decreased mean level of comet in treated groups compared to positive group was reported as shown in table (2) and fig.(5).

Table (1) : levels of brain tissue dopamine and caspase 3 in different studied groups

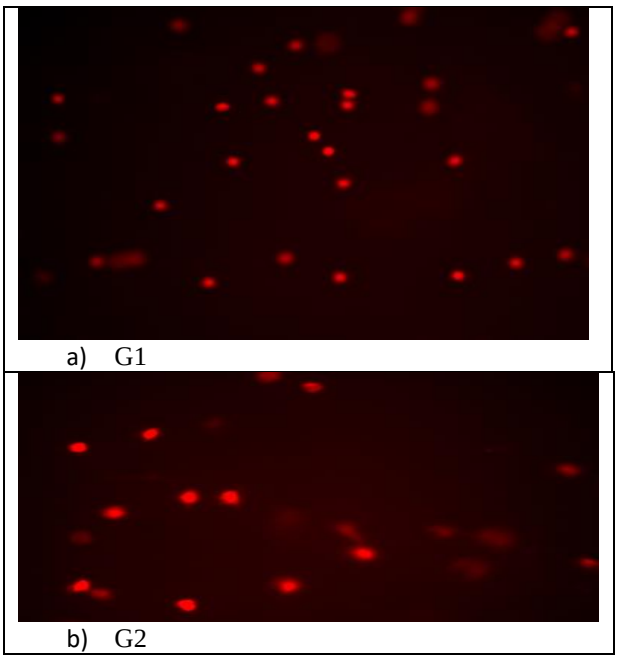
groups	Dopamine µg/g brain tissue	Caspase 3 ng/ml
G1	705 ± 30.9	9.5 ± 0.32
G2	591 ± 58.9	15.6 ± 1.02
G3	663 ± 42.8	11.37 ± 2.3
G4	698 ± 40.7	10.3 ± 0.79
G5	652 ± 19.5	11.35 ± 0.46
G6	713 ± 53.2	10.1 ± 0.83
G7	730 ± 41.2	9.8 ± 0.79

G1(Negative control group)-G2(Positive control group)-G3(Treated group with Punica granatum extract-G4(Treated group with Persea Americana extract)-G5(Ttreated group with reference drug aricept)-G6(Negative control+Punica granatum extract)-G7(Negative control+ Persea Americana extract).

Table (2) : comet assay % in whole blood of different studied groups

Groups	Comet %
G1	4.55±0.4
G2	58.17±9.08
G3	34.67±3.07
G4	30.33±3.5
G5	32.67±3.82
G6	4.07±0.57
G7	4.0±0.43

G1(Negative control group)-G2(Positive control group)-G3(Treated group with Punica granatum extract-G4(Treated group with Persea Americana extract)-G5(Ttreated group with reference drug aricept)-G6(Negative control+Punica granatum extract)-G7(Negative control+ Persea Americana extract).



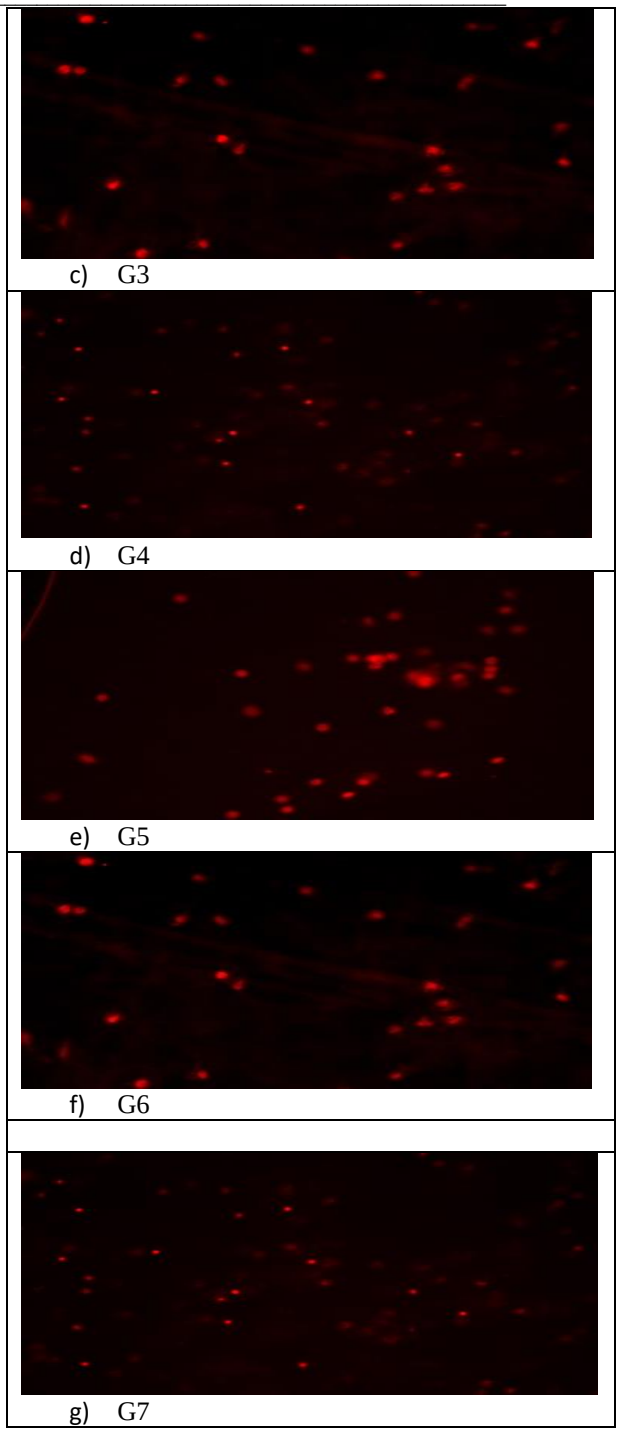


Fig.(5):a) G1 Negative control group- b) G2 Positive control group- c) G3 Treated group with Punica granatum extract- d) G4 Treated group with Persea Americana extract- e) G5 Treated group with reference drug aricept- f) G6 Negative control+Punicagranatum extract- g) G7 Negative control+Persea Americana extract

Histological investigation Fig.(6)

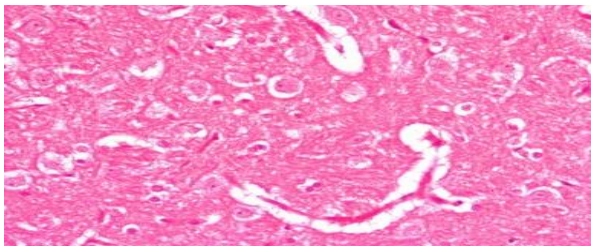


Fig.(6a) G1

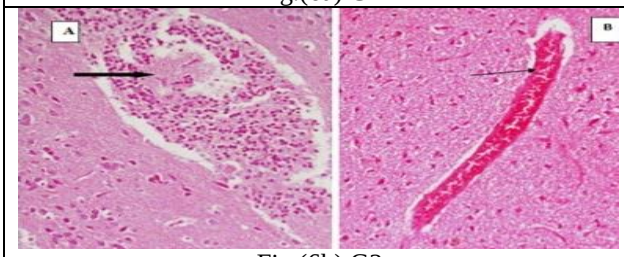


Fig.(6b) G2

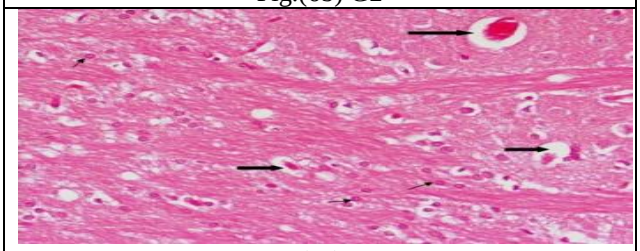


Fig.(6c) G3

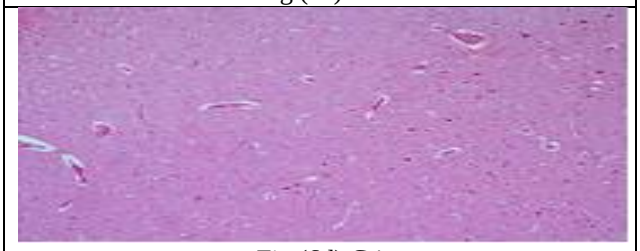


Fig.(6d) G4

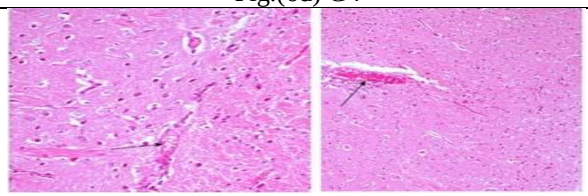


Fig.(6e) G5

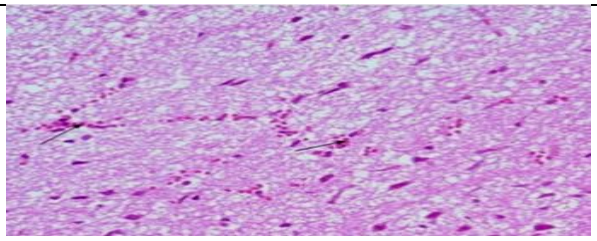
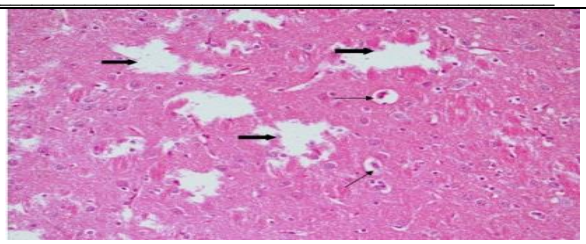


Fig.(6f) G6



Fig(6g) G7

Photomicrograph of (G1) negative control group showing normal neurons with round nuclei.(H&E400) as shown in fig(6a). Photomicrograph of (G2)positive control showing focal gliosis(A) and congested blood vessel (B) (H&E100) as shown in fig(6b). Photomicrograph of (G3) Punica granatum extract treated group showing increase number of mature cells (thin arrow) and few glial cells (thick arrow), with appearance of neuronal fibers and some vacuolated cells (H&E 200) as shown in fig(6c). Photomicrograph of (G4) Persea Americana extract treated group showing scattered glial cells, few numbers of mature neuronal cells(H&E 100) as shown in fig(6d)**.**Photomicrograph of(G5) referance drug(Aricept) group showing dilated congested vascular area (thin arrow) surrounded by glial and neuronal cells in neurofibrillary background (H&E100, 200) as shown in fig(6e). Photomicrograph of(G6) negative control + Punica granatum extract showing scattered hemorrhage(arrow), few neuronal cells and vacuolated background (H&E200) as shown in fig(6f). Photomicrograph of(G7) negative control + Persea americana extract showing large areas of hemorrhage (star), thickened arterial wall (thin arrow), plaque area (thick area) and few neuronal cells at the periphery (H&E 100) (H&E 200) as shown in fig(2g).

4-Discussion

Alzheimer's disease is a chronic, degenerative disease for which there was no cure until recently. Current therapy options slow the advancement of the disease, but they are costly and can induce unpleasant side effects in patients. Functional foods are a promising topic of study that is currently garnering attention for their potential to prevent and/or treat a variety of disorders, including neurodegenerative diseases [49]. As a result, the current study's aim was to investigate whether traditional herbs like Punica granatum and Persea americana extracts can reduce the progression of Alzheimer's disease in experimental animals caused by $AlCl_3$.The present study results showed that there was a reduction in the mean level of dopamine in brain tissue in positive control animal group compared to negative control. This finding could be related to a variety of factors that have been linked to brain injury, including the existence of extracellular amyloid protein deposits, senile plaques, and intracellular fibrillary tangles. All of these variables contribute to synapse disorganisation,

cell death, and neurotransmission dysfunction. Disruption of the dopaminergic system has been linked to the pathogenesis of Alzheimer's disease [50]. This result was in agreement with a study done by[51] who found that exposing male mice to aluminium chloride lowered dopamine levels compared to the control group.

Also, the study results revealed that there was a significant raising in the mean level of dopamine in brain tissue in treated groups with punica granatum and persea americana compared to positive group.

This result might be due to the antioxidants in punica granatum may be able to reverse the neurotransmitter restricting impact of AlCl3 exposure[52]. The significance of genistein and chickpea extract in enhancing the oxidative environment and the resistance to Al toxicity in male rats was explained in a study conducted by [53]. . Moreover, [54] emphasised the neuroprotective effect of curcumin polyphenols against motor and behavioural abnormalities caused by AlCl3 exposure in rats. [55] reported Persea americana species were contained high level of dopamine. Supplementing mice's diets with n-3 PUFAs has been shown to restore dopamine (DA) metabolism and normalise brain DA levels[56].

There was an increase in the mean level of dopamine in brain tissue in Aricept-treated groups because cholinesterase inhibitors (such as donepezil, galantamine, and rivastigmine) reversibly bind enzyme and limit the breakdown of acetylcholine in order to improve cholinergic neurotransmission. Acetylcholine is a critical neurotransmitter in the nervous system that interacts with receptors involved in learning and memory processes[57], hence an increase in ACh levels has been demonstrated to cause DA release in the striatum [58,59].

In addition, the current study found a significant rise in the mean level of caspase 3 in brain tissue in the positive control group compared to the negative control group. This result could be attributed to aluminum-induced brain toxicity and apoptosis, which results in a rise in caspase 3 levels in brain tissue[60].

Also, the present study results indicated that there was significant decreased in the mean level of caspase 3 in brain tissue in treated groups with punica granatum, persea americana and aricept and this result might be due to the antioxidant activities of the active constituents of the Punica granatum [61]**.** This result agreed with a study done by[62] who stated that the treated group's brain caspase 3 level was significantly lower than the control group's. Punica granatum extract may induce down-regulation of caspase-3 expression, agreed with [63] who suggested that Persea americana may have neuroprotective effects, such as mitigation of changes induced by psychosocial stress that lead to persistent brain abnormalities, and they concurred with [64], who suggested that aricept inhibited caspase-3 expression.

Furthermore, Al-induction caused significant DNA damage, as evidenced by increased DNA fragmentation and the number of comets visible on agarose gel electrophoresis, suggesting genotoxicity in the Al-induced cell compared to the control cell. Al neurotoxicity has previously been linked to DNA fragmentation and an increase in comets [65]. Furthermore, the current discovery is consistent with that of [66], who found that Al neurotoxicity can cause quicker neuronal apoptosis, as evidenced by micrographs that clearly demonstrated DNA damage and cell disintegration. Al's chemical nature as a trivalent cation with a high attraction for negatively charged groups like phosphates and phosphorylated proteins in nucleic acids could explain this oxidative DNA damage. As a result, Al may bind to DNA and RNA, impair enzyme activity, increase lipid peroxidative damage, and lower antioxidant status in the rat brain [67]. Also, the current study results founded that there was a significant decreased in the mean level of comet in whole blood of treated group with Punica granatum and Persea Americana compared to positive group. This could be because ellagic acid decreased Aβ assiciated cell death, lactate dehydrogenase membrane damage, DNA impairment, apoptosis, and the formation of reactive oxygen species (ROS). As a result, it has been postulated that ellagic acid can hydrogen link to the polar head groups of membrane phopholipids, protecting the cell membrane from oxidative stress [68]. and Several clinical investigations suggest that xanthophylls, which are comparable to those present in avocados, may have antioxidant and DNA-protective properties, as well as potential anti-aging benefits. In contrast to beta-carotene, lutein and oxidative DNA damage as determined by the comet assay were found to have inverse associations in other studies[69]. This result agreed with [70] who showed that P.americana reduced comet length. Flavonoids are thought to lessen the severity of DNA damage by intercalating themselves into DNA double helices, so stabilising DNA structure against free radical attack, according to several theories.

5-Conclusion

Alzheimer's disease caused by $AlCl_3$. Al may cause neurodegenerative illnesses in rats by activating caspases and causing genomic DNA damage in the brain, as well as a decrease in dopamine function. It also triggered apoptosis and cell death. Our findings showed that Punica granatum and Persea Americana extracts could be used as an agent for treating neurodegenerative effects of $AlCl_3$-induced neurological dysfunction by reducing the negative effects of $AlCl_3$ on the majority of the parameters studied. Punica granatum and Persea Americana are

powerful antioxidants that boost antioxidant status and prevent oxidative damage.

6-Conflicts of interest

There are no conflicts to declare

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7-References

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