The Anti-neurological Activity of Pomegranate (*Punica granatum* L.) (*Punica granatum* L.) and *Annona Muricata* Linn Pulp Fruit juice in Aluminum Chloride Induced Rats Model

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Abstract

Pomegranate (*Punica granatum* L.) (*Punica granatum* L.) and *Annona Muricata* fruit juices are rich in bioactive compounds with potent antioxidant and neuroprotective properties. Therefore, this Study was aimed to investigate the anti-neurological disease activity of Pomegranate (Punica granatum L.) and *Annona Muricata* fruit pulp juice in an aluminum chloride induced rat model. Fifty adult male rats were randomly divided into five equal groups (n = 10 each). The first group (negative control) was fed on basal diet only. The groups were injected with aluminum chloride through intraperitoneal (i.p.) route at a dose of 4.2 mg/kg body weight for 28 ($Alcl_3$) to induce neurodegeneration (ND), the second group (positive control) received a basal diet. The groups from (3 to 5) received a basal diet and given orally a Pomegranate (*Punica granatum* L.) juice (PJ) (*Punica granatum*), *Annona Muricata* juice (AMJ) and mixture of PJ and AMJ (1:1 v/v) (1 ml/100 g body weight) daily, respectively for one months. Moreover, neurochemical parameters, including acetylcholine concentration, β-hydroxybutyrate, amyloid beta, and hyperphosphorylated tau protein (p-tau) in serum, showed marked ($P<0.05$) improvement, suggesting a neuroprotective effect. It was also observed that the mixture group exhibiting the most pronounced effects indicating a relatively stronger capacity to modulate tau hyperphosphorylation and mitigate aluminum-induced neuronal damage. So that, these findings support their potential therapeutic application in the prevention and management of neurodegenerative disorders

Keywords: Pomegranate (*Punica granatum* L.), *Annona Muricata*, Aluminum chloride, Neurodegeneration, Antioxidant, Neuroprotection, Oxidative stress, Amyloid beta, Hyper phosphorylated tau, Acetylcholine, Rats.

Introduction

Neurological diseases such as Alzheimer's disease, Parkinson's disease, stroke, epilepsy, traumatic brain injury, amyotrophic lateral sclerosis and multiple sclerosis pose a great challenge to healthcare systems and rank as the leading cause of mortality and disability worldwide **(Feigin *et al.,* 2020).**

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative illness first defined by a germane psychiatrist Alois Alzheimer in 1906 characterized by impairment of short and long memory, abnormal behavior, disorientation, deterioration of motor skills, sleeping disturbance, and impairment of cognitive functions leading to disability to carry on simple daily tasks followed by total dependence on caregivers. AD is considered the sixth leading cause of death **Kumar *et al*., (2022)**.

Many theories have been implicated in the progression of AD, including the dysfunctionality of the acetylcholine neurotransmitter **(Francis,2005)**, oxidative stress **Cassidy *et al*., (2020)**, inflammation **Kinney *et al*., (2018)**, and hyperglycemia **Lee *et al*., (2018)**. Also, there are many pathognomonic lesions associated with AD, such as amyloid plaques **Deture *et al*., (2019)**, hippocampus degeneration **Rao *et al*., (2022)** and cerebral cortex shrinkage **Ramos *et al*., (2017)**.

Continuous exposure to heavy metals has multiple negative impacts on human health. One of the widely used heavy metals is Aluminum, which is used in foils, utensils **Kinawy, (2019)**, food additives, beverages, toothpaste, paper making **Praveenkumar *et al*., (2019)**. Recently, Aluminum has been known to selectively accumulate in the brain leading to AD by many mechanisms, including oxidative stress, synapse dysfunction, apoptosis induction, proinflammatory cytokines and cyclooxygenases overproduction,

microgliosis, promoting amyloid, tau, aggregation and altering neurotransmitter metabolism **Skalny *et al*., (2021)**.

Natural compounds gained attention for their intrinsic multifunctional nature, and semisynthetic and fully synthetic molecules inspired by natural compounds have been largely studied for their potential effects against AD and neurodegenerative diseases **Lueptow *et al*., (2017) and Liu *et al*., (2017)**. Pomegranate (Punica granatum L.) fruit (*Punica granatum L*.) has been recognized as some fruit rich in bioactive molecules. It has been extensively used in traditional medicine, such as the Pomegranate (Punica granatum L.) fruit is one of the fruits that contains the highest amount of polyphenols such as anthocyanins, tannins, flavonoids, phenolic acids, and lignans **Huang *et al*., (2021)**.

The nutraceuticals are contained both in the edible part of the fruit (pulp and seeds) and in the peel, leaves, flowers, and hull, the processing by-products of the plant **Wang *et al*., (2017) and Wang *et al.,* (2016)**. Recently, several studies reported that the bioactive molecules contained in Pomegranate (Punica granatum L.) seed, Pomegranate (Punica granatum L.) peel, and Pomegranate (Punica granatum L.) juice have a potential beneficial role in AD against the formation of ROS, reducing neuroinflammation, inhibiting AChE, and decreasing the Aβplaques and NFTs **Chang *et al*., (2019)**.

Annona muricata Linn. (Annonaceae) fruit is commonly known as “Soursop” or “Graviola” It is a terrestrial deciduous tree and produces an edible fruit. This species of Annona has been grouped with the “cherimoya” plants of the Annonaceae family. Although this Annonacea is native to America, it has now become established in many tropical countries. The (*Annona muricata* L.) is a plant species used in Mexican traditional medicine, where the leaves are used to treat various diseases, such as stomach pain, bronchitis and gastric cancer, and it has even been called a “cancer killer” **Adewole *et al*., (2008)** and **Jacobo *et al*., (2016**).

In Africa, Asia and South America, the seeds of this species of Annona are used to treat several types of cancer due to the chemo preventive properties presented by *A. muricata* extracts, which can induce apoptosis of cancer cells, along with the synergistic effects of polyphenols, flavonoids, alkaloids and lipophilic antioxidant compounds **León *et al*., (2015)**.

Also, it is well known that the species of the Annonaceae family besides producing polyphenols, flavonoids and alkaloids, produces large quantities of acetogenins, a secondary metabolite derived from long-chain fatty acids that have been found to have anticancer, anti-inflammatory, antibacterial and antiviral properties **Pieme *et al*., (2014)** and the phenolic compounds in A. muricata, such as quercetin and gallic acid, are reported to be the compounds most responsible for the antioxidant capacity of the plant **Biba *et al*., (2014).** Therefore, this work was amid to investigate the Anti-neurological Activity of Pomegranate (Punica granatum L.) and *Annona Muricata* Linn Pulp Fruit juice in Aluminum Chloride Induced Rat Model

Materials and methods

Materials:

Fully ripe fresh Pomegranate (*Punica granatum* L.) and *Annona Muricata* were obtained from the local market and were identified at the Agriculture Research Centre. Fifty-three adult male rats (Strain), weighing 200±5g, were obtained from the Laboratory Animal Colony, Helwan, Egypt. Casein, cellulose, choline chloride, D-L methionine, vitamins and minerals, constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soybean oil and sucrose were obtained from the local market. Chemicals and Kits for biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemical, Dokki, Egypt.

Methods:

1. **The taxonomic classification of:**

A. Pomegranate (*Punica granatum* L.): Kingdom: Plantae; **Division**: Magnoliophyta; **Class**: Magnoliopsida; **Order**: Myrtales; **Family:** Punicaceae; **Genus**: Punica; Species: P. granatum or P. protopunica

B. *Annona Muricata* linn**: Kingdom**: Plantae; **Division**: Magnoliophyta; **Class**: Magnoliopsida; **Order:** Magnoliales; **Family**: Annonaceae; **Genus**: Annona L.; **Species**: *Annona Muricata L.*

2. **Chemical composition:** The Chemical composition and the active ingredients of the tested fruits were determined according to the official methods **(AOAC, 2019).** Total phenolic and total flavonoid content were expressed as mg of gallic acid equivalent (GAE) per g of sample determined according to the procedure of **(Zilic *et al*., 2012)**. The total flavonoid content of Pomegranate (Punica granatum L.) and *Annona muricata* (soursop) juices was determined by the method of **Kim *et al.*, (2003).** The DPPH radical scavenging activity of these juices was assessed as described by **Blois, (1958).**
3. **Preparation of Basal Diet:** The basal diet (AIN-93M) consisted of protein (14%), corn oil (**4**%), minerals mixture (3.5%), vitamins mixture (1%), fiber (5%), sucrose (10%), choline chloride (0.25%) and the remainder was corn starch up to 100%. These constituents were thoroughly mixed and formulated according to **Reeves *et al.*, (1993).**
4. **Preparation of Fruits juice**: One kilogram of Pomegranate (Punica granatum L.) seeds and/or Annona pulp fruit were blended, filtered through a double layer of gauze to obtain fruit juices and kept in a refrigerator till use (2-5 0C) **(Asanga et al., 2015).**
5. **Induction of Neurological Disease (ND):** Aluminum chloride was dissolved in water and injected to rats through intraperitoneal (i.p.) route at a dose of 4.2 mg/kg body weight lightly

anaesthetized with ether. Aluminum chloride was given for 28 days as stated by **Bitra *et al*., (2014).** To ensure the induction of Neurological Disease, random blood samples were taken from three rats to determine amyloid beta and tau protein concentration in serum.

The Biological study: All rats were housed at a room temperature of 25 ± 2 °C, relative humidity of 50–55% and 12 hr. light/12 hr. dark cycles in the animal house of the Faculty of Home Economics, Cairo, Egypt for one week for acclimatization. Fifty adult male rats were randomly divided into five equal groups (n = 10 each). The first group (negative control) was fed on basal diet only. The other rats were injected with aluminum chloride through intraperitoneal (i.p.) route at a dose of 4.2 mg/kg body weight for 28 ($Alcl_3$) to induce neurodegeneration (ND), the second group (positive control) received a basal diet. The groups from (3 to 5) received a basal diet and given orally a Pomegranate (*Punica granatum* L.) juice (PJ***) (Punica granatum),*** *Annona Muricata* juice (AMJ) and mixture of PJ and AMJ (1:1 v/v) (1 ml/100 g body weight) daily, respectively for one months.

At the end of the experimental period (one month), rats were fasted for 12 hours then sacrificed. Blood samples were collected from the portal vein into dry clean centrifuge tubes. Serum was separated by centrifuge at 3000 r.p.m for 15 min and serum aliquots were stored at –20ºC until use for biochemical analysis.

6. **Biochemical analysis:** All of the biochemical assays were carried out at the National Research Center, Cairo, Egypt.

Amyloid beta concentration in brain was determined according to **(Bittner et al., 2016)**. Hyperphosphorylated tau protein (p-tau) according to (**Lifke et al., 2019)**. Serum B-hydroxybutyrate concentration and Acetylcholine (ACH) concentration were determined according to **Rozga et al., (2017).**

7. Statistical analysis: The obtained results were expressed as Mean ± SE. Data were evaluated statistically with computerized SPSS package program (SPSS 22.00 software for Windows) using one-way analysis of variance (ANOVA). A significant difference among means was estimated at $p<0.05$ (**Sendcor and Cochran, 1979).**

Results and Discussions

Table (1) presents the chemical composition and nutritional profile of Pomegranate (*Punica granatum* L.) juice and *Annona muricata* fruit juice. The moisture content was high in both juices, reaching (85.55 g/100 mL) in Pomegranate (Punica granatum L.) and (81 g/100 mL) in *Annona muricata*, reflecting their high water content. Protein and fat contents were relatively low in both juices, with *Annona muricata* showing higher values (1.02 g/100 mL) protein and (0.31 g/100 mL) fat compared to Pomegranate (*Punica granatum* L.) (0.14 g/100 mL) protein and (0.04 g/100 mL) fat. Ash content was also slightly higher in *Annona muricata* (0.50 g/100 mL) than in Pomegranate (*Punica granatum* L.) (0.33 g/100 mL). Dietary fiber was measured at (2.79 g/100 mL) in Pomegranate (*Punica granatum* L.) juice and (3.28 g/100 mL) in *Annona muricata* juice, while carbohydrate content was higher in *Annona muricata* (16.8 g/100 mL) compared to Pomegranate (Punica granatum L.) (11.15 g/100 mL).

Both juices contained significant amounts of bioactive compounds. Total phenolic content was highest in Pomegranate (*Punica granatum* L.) (780 mg GAE/100 mL) while *Annona muricata* contained (186 mg GAE/100 mL). Total flavonoid content was (12.1 mg CE/100 mL) in Pomegranate (Punica granatum L.), whereas *Annona muricata* had (87.17 mg quercetin/100g). The DPPH radical scavenging activity, which reflects antioxidant

potential, was (47.38%) for Pomegranate (*Punica granatum* L.) and (32.60%) for *Annona muricata*. Vitamin C content was higher in *Annona muricata* (29.6 mg/100 mL) than in Pomegranate (*Punica granatum* L.) (6.09 mg/100 mL), further supporting its nutritional value and antioxidant activity. These results highlight the nutritional and antioxidant potential of both Pomegranate (*Punica granatum* L.) and *Annona muricata* juices, supporting their inclusion as health-promoting beverages in the diet.

The results of chemical composition of Pomegranate (*Punica granatum* L.) juice are harmony with **(Li et al., 2006; Jahfar et al., 2003 and Tezcan et al., 2009).** On the other hand, the results of Annona muricata are harmony with **Jirovetz et al., (1998 and Gyesi et al., 2019)**

Table (1): Chemical composition of Pomegranate (*Punica granatum* L.) and *Annona Muricata* fruit juices

Component	Pomegranate (*Punica granatum* L.)	*Annona Muricata*
Moisture	85.55 (g/100ml)	81 (g/100ml)
Protein	0.14 (g/100ml)	1.02 (g/100ml)
Fat	0.04 (g/100ml)	0.31 (g/100ml)
Ash	0.33 (g/100ml)	0.50 (g/100ml)
Dietary fiber	2.79 (g/100ml)	3.28 (g/100ml)
Carbohydrate	11.15 (g/100ml)	16.8 (g/100ml)
Total phenolic content	780 (mg GAE/100 mL)	186 (mg GAE/100 mL)
Total flavonoid content	12.1 (mg CE/100 mL)	87.17 (mg quercetin/100g)

DPPH radical scavenging activity	47.38 %	32.60 %
Vitamin C	6.09 (mg/100 mL)	2٩.6 (mg/100 mL)

The effects of Pomegranate (*Punica granatum* L.) juice, *Annona Muricata* fruit juice, and their combination on β-hydroxybutyrate, in rats exposed to aluminum chloride were recorded at Table (2). The present study demonstrated that aluminum chloride caused a significant decreased β-hydroxybutyrate (β-HB) in rats, indicating enhanced lipid peroxidation and impaired mitochondrial energy metabolism. β-hydroxybutyrate, a biomarker inversely associated with oxidative stress and energy metabolism, was significantly decreased in the aluminum-exposed control group (22.70 ± 2.16) when compared to the normal rats (51.70 ± 2.04). This decline suggests impaired metabolic responses due to oxidative injury. Fruit juice treatments led to a dose-dependent improvement in β-hydroxybutyrate levels. The mixture group demonstrated the most pronounced elevation (41.93 ± 2.57), approaching normal values, while *Annona Muricata* and Pomegranate (*Punica granatum* L.) juices produced moderate increases (33.46 ± 1.30 and 31.23 ± 1.91, respectively), both of which were significantly higher than the untreated positive control ($p < 0.05$).

Table (2) reinforces the protective potential of both Pomegranate (Punica granatum L.) and *Annona Muricata* juices against aluminum-induced oxidative damage. Their combination consistently exerted a more substantial restorative effect on oxidative biomarkers, supporting the hypothesis of synergistic interactions that enhance antioxidant defense and energy metabolism more effectively than either juice administered alone.

These observations are supported by previous studies with clearly defined doses. **Akbarian *et al.,* (2023)** reported that administration of Pomegranate (*Punica granatum* L.) peel extract at 50 mg/kg/day improved antioxidant homeostasis and attenuated histopathological

changes in the hippocampus of aluminum chloride-induced Alzheimer's rats, consistent with the present findings. Annona muricata leaf extract at 100 mg/kg/day enhanced serum and urinary β-hydroxybutyrate levels in rats, supporting the protective effect observed in our study (**Ikenna-Ossai *et al.*, 2019)**.

Exposure to aluminum chloride (100 mg/kg body weight/day) in rats significantly decreased β-hydroxybutyrate and antioxidant enzyme activities, reflecting increased oxidative stress and impaired mitochondrial function, which aligns with the alterations observed in the present investigation (**Kumar and Gill, 2009**). **Exley, (2017)** also reported that aluminum exposure disrupts cellular bioenergetics and induces oxidative stress, corroborating the findings of increased lipid peroxidation and decreased β-HB levels seen here.

Table (2): The effect of Pomegranate (*Punica granatum* L.), *Annona Muricata* fruit juices and their mixtures on β-hydroxybutyrate in Aluminum Chloride Induced Rat Model

Groups \ Parameters		β-hydroxybutyrate (mmol/L)
-Ve Control group		51.70±2.04[a]
+Ve Control group		22.70±2.16[d]
Treated groups	**Pomegranate (Punica granatum L.) Juice**	31.23±1.91[c]
	***Annona Muricata* fruit Juice**	33.46±1.30[c]
	Mixture	41.93±2.57[b]

Data are expressed as mean ± SE

Means with different letters in each column are significantly differs at P<0.05

The positive control group, which injected with aluminum chloride without therapeutic intervention, exhibited a significant

increase in acetylcholine levels (165.36 ± 2.40 U/L) compared to the negative control group (100.16 ± 2.67 U/L), indicating a pronounced disruption of cholinergic function due to neurotoxic insult ($p < 0.05$) as seen at Table (3). Moreover, administration of Pomegranate (*Punica granatum* L.) juice led to a significant reduction in acetylcholine levels (132.96 ± 1.87 U/L) relative to the positive control, suggesting a protective effect potentially mediated by its rich content of polyphenolic compounds and antioxidants. Furthermore, a more notable decrease was observed in the group treated with *Annona Muricata* juice, which showed a further reduction to 123.56 ± 1.80 U/L, implying a comparatively stronger modulatory influence on cholinergic imbalance.

Interestingly, the group receiving the combined treatment of both juices demonstrated the greatest reduction in acetylcholine levels (117.12 ± 1.63 U/L), pointing to a potential synergistic interaction that enhanced the overall neuroprotective efficacy. Nevertheless, despite the improvements observed across all treated groups, acetylcholine concentrations remained significantly elevated when compared to the negative control group ($p < 0.05$), indicating partial restoration rather than full normalization of cholinergic function.

These findings further underscore the therapeutic potential of Pomegranate (*Punica granatum* L.) and *Annona Muricata* fruit juices in mitigating aluminum-induced neurotoxicity. The superior effect observed in the combination group highlights the possible advantage of utilizing a multi-component intervention targeting multiple neurochemical pathways. Such results warrant further investigation into the mechanisms of action and the clinical relevance of these natural products in the context of neurodegenerative disease prevention and management.

These findings are strongly supported by previous studies using clearly defined doses. **Abu-Taweel *et al.*, (2020)** administered Pomegranate juice at a 20% concentration to male mice exposed to aluminum chloride and reported significant improvements in

neurobehavioral and biochemical parameters, including normalization of acetylcholine (ACh) levels, consistent with the attenuation of ACh observed in the present study. On the other hand, **Uba *et al.,* (2024)** found that administration of Annona muricata leaf extract at 500 mg/kg/day significantly inhibited acetylcholinesterase activity and restored acetylcholine levels in aluminum lactate-induced Alzheimer's-like rats. In addition, Annona muricata seed extract at 200 mg/kg/day restored acetylcholine concentrations and improved neurological functions in aluminum chloride-exposed rats (**Ehiremen *et al.*, 2025)**, supporting the neuroprotective effect observed in our study.

Notably, combinations of these extracts have demonstrated synergistic effects. **El-Sayed *et al.,* (2021)** showed that co-administration of Pomegranate and Annona extracts at 200 mg/kg/day each synergistically improved cholinergic dysfunction and reduced oxidative stress markers in aluminum chloride-treated rats. Likewise, **Chen *et al.,* (2023)** reported that polyphenol-rich fruit extracts at 150 mg/kg/day normalized neurotransmitter balance and decreased cholinergic disruption, consistent with the pronounced normalization of ACh levels observed in our mixture-treated group.

Table (3): The effect of Pomegranate (*Punica granatum* L.), *Annona Muricata* fruit juices and their mixtures on Acetylcholine concentrations in Aluminum Chloride Induced Rat Model

Parameters / Groups		Acetylcholine (U/L)
-Ve Control group		100.16±2.67^{e}
+Ve Control group		165.36±2.40^{a}
Treated groups	**Pomegranate (Punica granatum L.) Juice**	132.96±1.87^{b}
	***Annona Muricata* fruit Juice**	123.56±1.80^{c}
	Mixture	117.12±1.63^{d}

Data are expressed as mean ± SE

Means with different letters in each column are significantly differs at $p<0.05$

Remarkably, the group receiving the combined juice treatment exhibited the lowest p-tau concentration among the treated groups (9.86 ± 0.51), approaching levels observed in the negative control. This notable outcome implies a potential synergistic interaction between the bioactive compounds present in both juices, enhancing their collective efficacy in suppressing tau pathology and preserving neuronal function. Despite the observed improvements, p-tau levels in all treated groups remained significantly higher than those of the negative control group ($p < 0.05$), indicating that while the treatments offered partial neuroprotection, they did not achieve complete normalization.

Collectively, these findings underscore the therapeutic potential of Pomegranate (*Punica granatum* L.) and *Annona Muricata* juices—particularly when combined—as natural agents capable of attenuating tau-associated neurotoxicity. Further investigation is warranted to elucidate their mechanisms of action and to assess their clinical relevance in the prevention or management of tau-related neurodegenerative disorders such as Alzheimer's disease.

These findings are supported by recent studies using well-defined doses of natural extracts in aluminum-induced neurotoxicity models. **Harakeh *et al.,* (2020)** reported that Pomegranate peel extract at 50 mg/kg/day alleviated histopathological alterations and restored antioxidant homeostasis in the hippocampus of aluminum chloride-treated rats, consistent with the reduction in p-tau observed in the PJ-treated group in the present study. Annona muricata seed extract at 100 mg/kg/day to aluminum chloride-induced Alzheimer's disease rats. The treatment significantly reduced oxidative stress markers and histopathological alterations in the hippocampus (**Ehimare *et al.*, 2025)**, supporting the reduction in hyperphosphorylated tau protein (p-tau) observed in the AMJ-treated group in the present study.

Importantly, the mixture-treated group exhibited the lowest p-tau levels, approaching those of the negative control group, reflecting a synergistic or additive effect and confirming that the combination is more effective than either extract alone. Furthermore, **Alami *et al.*, (2024)** provided mechanistic evidence that Pomegranate-derived polyphenols—including peel, aril, punicalagin, and ellagic acid—attenuated tau phosphorylation (p-Tau-181), neuroinflammation, and oxidative stress in human neuronal models challenged with amyloid-β, supporting the modulatory effects on tau pathology observed in the mixture-treated group in our study. Combinations of natural extracts, including Pomegranate and Annona, demonstrated significant reductions in tau phosphorylation, oxidative stress, and neuroinflammation (**Ojetunde, 2024)**, supporting the synergistic effects observed in our study.

Table (4): The effect of Pomegranate (*Punica granatum* L.), *Annona Muricata* fruit juices and their mixtures on brain tissues Hyperphosphorylated tau protein in Aluminum Chloride Induced Rat Model

Parameters / Groups		Hyperphosphorylated tau protein (p-tau)
-Ve Control group		7.63±0.24^{e}
+Ve Control group		22.70±1.30^{a}
Treated groups	**Pomegranate (Punica granatum L.) Juice**	16.36±0.41^{b}
	***Annona Muricata* fruit Juice**	12.83±0.68^{c}
	Mixture	9.86±0.51^{d}

Data are expressed as mean ± SE

Means with different letters in each column are significantly differs at $p<0.05$.

The results presented in **Table (4)** indicate the impact of Pomegranate (*Punica granatum* L.) juice, *Annona Muricata* fruit juice, and their combination on hyperphosphorylated tau protein (p-tau) levels in brain tissues of rats exposed to aluminum chloride-induced neurotoxicity. As expected, administration of aluminum chloride (positive control group) resulted in a substantial elevation in p-tau levels (22.70 ± 1.30) compared to the negative control group (7.63 ± 0.24), reflecting a marked disruption in tau protein homeostasis and a potential onset of tauopathy ($p < 0.05$).

Treatment with Pomegranate (*Punica granatum* L.) juice significantly reduced p-tau levels to 16.36 ± 0.41, suggesting a neuroprotective effect, likely attributed to its rich polyphenolic content and antioxidant activity, which may counteract oxidative stress and downregulate abnormal phosphorylation signaling pathways. A more pronounced reduction was observed in rats treated with *Annona Muricata* juice (12.83 ± 0.68), indicating a relatively stronger capacity to modulate tau hyperphosphorylation and mitigate aluminum-induced neuronal damage.

The neurotoxic insult significantly elevated Aβ levels in the positive control group, with serum concentrations reaching 247.90 ± 2.56 pg/ml and brain concentrations rising to 129.13 ± 2.54 ng/mg. These values markedly exceeded those observed in the negative control group (137.23 ± 3.83 pg/ml and 78.43 ± 2.49 ng/mg, respectively), confirming the amyloidogenic potential of aluminum exposure ($p < 0.05$), as observed at **Table (5)**.

Administration of Pomegranate (*Punica granatum* L.) juice resulted in a significant reduction in Aβ levels (201.76 ± 2.27 pg/ml in serum and 112.00 ± 1.73 ng/mg in brain), suggesting a moderate modulatory effect, likely attributable to its antioxidant polyphenols and their regulatory role in amyloid precursor protein (APP) processing and Aβ aggregation. In contrast, rats receiving *Annona Muricata* juice exhibited a more substantial decrease in both serum

and brain Aβ concentrations (185.60 ± 1.79 pg/ml and 103.60 ± 2.53 ng/mg, respectively), highlighting a potentially more effective inhibition of amyloidogenic pathways. Notably, the combination therapy produced the most pronounced effect, with Aβ concentrations falling to 166.40 ± 2.06 pg/ml in serum and 89.33 ± 3.43 ng/mg in brain tissues. This enhanced efficacy may reflect a synergistic interaction between the bioactive phytochemicals of both juices, leading to improved regulation of amyloid clearance mechanisms and a stronger neuroprotective profile.

Although none of the treatments fully normalized Aβ levels to those of the negative control group ($p < 0.05$), the reductions achieved were biologically significant and indicative of partial therapeutic benefit. Taken together, the results underscore the potential of using Pomegranate (*Punica granatum* L.) and *Annona Muricata* juices—particularly in combination—as functional nutritional strategies to combat amyloid beta accumulation, a central hallmark of Alzheimer's disease. These outcomes are consistent with growing preclinical evidence supporting the efficacy of plant-derived bioactives in the modulation of amyloid pathology within the emerging discipline of nutritional neuroscience.

The present study demonstrated that the mixture produced the most pronounced reduction in Aβ levels, suggesting a synergistic neuroprotective effect of the bioactive compounds present in both juices. Recent studies have provided compelling evidence supporting the neuroprotective effects of Pomegranate (*Punica granatum* L.) juice (PJ) in aluminum chloride-induced neurotoxicity models. Pomegranate (*Punica granatum* L.) juice at a 20% concentration to male mice exposed to aluminum chloride resulted in a significant reduction in amyloid-beta (Aβ) accumulation and improvement in neurobehavioral outcomes (**Abu-Taweel *et al.*, 2021)**, which aligns with the reduction observed in the PJ-treated group in the present study. Pomegranate (*Punica granatum* L.) seed oil enhanced memory by reducing oxidative stress and Aβ accumulation in transgenic mice (**Chatzikostopoulos *et al.*, 2024)**.

Annona muricata seed extract at 200 mg/kg/day reduced Amyloid beta accumulation and improved inflammatory markers in an Alzheimer's disease rat model (**Ehimare *et al.*, 2025),** consistent with the modulatory effect of AMJ observed here. Similarly, ***Uba et al.,*** **(2024)** administered Annona muricata leaf extract at 500 mg/kg/day to aluminum lactate-induced Alzheimer's-like rats. The treatment significantly decreased amyloid-beta accumulation in the brain and improved neurological function, corroborating the reductions in serum and brain amyloid-beta levels observed in the AMJ-treated group and aligning with the modulatory impact of the juice mixture observed in the present study.

The combination of both extracts at 200–450 mg/kg/day each significantly reduced Aβ deposition in aluminum-exposed rats **(Folami *et al.,* 2024)**, consistent with the pronounced reduction of Aβ observed in our mixture-treated group.

Table (5): The effect of Pomegranate (*Punica granatum* L.), *Annona Muricata* fruit juices and their mixtures on serum Amyloid beta and Amyloid beta in brain in Aluminum Chloride Induced Rat Model

Parameters / Groups		Amyloid beta (Pg/ml)	Amyloid beta in brai (ng/mg tissue)
-Ve Control group		137.23±3.83^{e}	78.43±2.49^{e}
+Ve Control group		247.90±2.56^{a}	129.13±2.54^{a}
Treate groups	Pomegranate (Punica granatu L.) Juice	201.76±2.27^{b}	112.00±1.73^{b}
	Annona Muricata fruit Juice	185.60±1.79^{c}	103.60±2.53^{c}
	Mixture	166.40±2.06^{d}	89.33±3.43^{d}

Data are expressed as mean ± SE

Means with different letters in each column are significantly differs at $p<0.05$

Collectively, these studies confirm that PJ and AMJ, individually and in combination, at clearly defined doses, can effectively reduce amyloid beta accumulation, supporting their potential role in mitigating aluminum-induced amyloidogenic and neurodegenerative alterations. So that, these findings support their potential therapeutic application in the prevention and management of neurodegenerative disorders

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الملخص

تُعد عصائر فاكهة الرمان والقشطة مصادر غنية بالمركبات الحيوية النشطة التي تمتاز بخصائص مضادة للأكسدة وحامية للأعصاب. هدفت هذه الدراسة إلى تقييم التأثير المضاد للأمراض العصبية لعصير لب الرمان وعصير فاكهة القشطة في نموذج الفئران المُستحث بكلوريد الألمنيوم. تم تقسيم خمسين فأرًا ذكرًا بالغًا بشكل عشوائي إلى خمس مجموعات متساوية (١٠ فئران لكل مجموعة). تلقت المجموعة الأولى (الضابطة السالبة) النظام الغذائي الأساسي فقط، بينما تم حقن المجموعات الأخرى في الغشاء البريتوني بكلوريد الألومنيوم (4.2)٤,٢ مللي/كجم من وزن الجسم لمدة ٢٨ يوم لتحفيز حدوث تلف الخلايا العصبي، حيث تلقت المجموعة الثانية (الضابطة الموجبة) النظام الغذائي الأساسي فقط. أما المجموعات من الثالثة إلى الخامسة، فقد تم تغذيتها بالنظام الغذائي الأساسي مع اعطاء عصير الرمان، وعصير فاكهة القشطة، ومزيج منهما بنسبة (1:1) (v/v)عن طريق الفم (١ مل لكل ١٠٠ جم من وزن الجسم)، على التوالي لمدة شهر. أظهرت النتائج تحسنًا معنويًا في المؤشرات العصبية، بما في ذلك تركيز الأستيل كولين، والبيتا-هيدروكسي بيوتيرات، والبيتا أميلويد، وبروتين تاو الفائق الفسفرة (p-tau) في أنسجة الدماغ، ولوحظ أيضا أن مجموعة الخليط التي تظهر التأثيرات الأكثر وضوحا مما يشير إلى قدرة أقوى نسبيا على تعديل فرط الفسفرة والتخفيف من تلف الخلايا العصبية الناجم عن الألومنيوم .بحيث تدعم هذه النتائج تطبيقها العلاجي المحتمل في الوقاية من الاضطرابات التنكسية العصبية.

الكلمات المفتاحية: الرمان، فاكهة القشطة ، كلوريد الألومنيوم، التحلل العصبي، مضادات الأكسدة، الحماية العصبية، الإجهاد التأكسدي، بيتا أميلويد، تاو عالي الفوسفور، الأسيتيل كولين، الفئران.