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Antioxidant Potential And Neuroactive Properties Of Malus Domestica: An In-Vitro DPPH Assay And GABA Modulation Clinical Study

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Abstract

Background: *Malus domestica (apple) is widely recognized for its rich phytochemical composition and associated health benefits, yet its combined antioxidant potential and neuroactive effects remain underexplored.*

Objective: *This study evaluated the in vitro antioxidant activity of Malus domestica extracts and assessed their potential neuroactive effects through modulation of plasma γ-aminobutyric acid (GABA) levels in a clinical pilot study.Methods: Methanolic extracts of apple powder were analyzed for phytochemical content and tested using the DPPH radical scavenging assay at concentrations ranging from 50–400 µg/mL. For the clinical component, 40 healthy adults participated in an 8-week randomized, placebo-controlled trial; plasma GABA levels were measured pre- and post-intervention using ELISA. Statistical analyses included descriptive statistics, normality testing, paired comparisons, and correlation analysis.*

Results: *The apple extract demonstrated strong dose-dependent antioxidant activity, with mean % inhibition of approximately 58.9% and IC_{50} consistent with reported values for polyphenol-rich extracts. Clinically, participants receiving the apple extract showed a modest increase in mean plasma GABA levels (from 6.50 ng/mL to 7.15 ng/mL). Correlation analysis revealed a moderate positive relationship between baseline and post-intervention GABA levels ($r = 0.565$, $p = 0.035$), though changes in GABA were not significantly correlated with IC_{50} values ($r = 0.220$, $p = 0.449$).*

Conclusion: *Malus domestica exhibits notable in vitro antioxidant activity and potential to modestly enhance plasma GABA levels, suggesting neuroactive properties. Further large-scale studies are warranted to confirm these findings and elucidate underlying mechanisms.*

Keywords *Malus domestica, Antioxidant activity, DPPH assay, GABA modulation, Neuroactive properties.*

1. INTRODUCTION

Apples (Malus domestica) are among the most widely cultivated and consumed fruits worldwide, valued not only for their nutritional content but also for their rich array of bioactive phytochemicals. These compounds including flavonoids (such as quercetin and rutin), phenolic acids, triterpenoids, and polyphenols are distributed throughout the peel, flesh, and seeds of the fruit and have been shown to possess significant antioxidant, anti-inflammatory, and neuroprotective properties[1][2]. Recent scientific interest has focused on the role of apple-derived phytochemicals in neurological health, as emerging evidence suggests these compounds can influence brain function, protect neuron cells against oxidative stress-induced neurotoxicity, and promote adult neurogenesis, particularly in the hippocampus a brain region critical for learning and memory[3][4].

The mechanistic insights into how these phytochemicals exert their effects include modulation of molecular signalling pathways involved in oxidative stress response, neuroinflammation, and neuronal survival and differentiation[5]. As neurodegenerative diseases and cognitive decline become increasingly prevalent with aging populations, understanding the therapeutic potential of Malus domestica phytochemicals in neurological health has become a promising area of research. This paper aims to provide a comprehensive overview of the phytochemical profile of apples, elucidate their mechanisms of action in the nervous system, evaluate their therapeutic prospects, and discuss the current state of clinical research in this rapidly evolving field[6].

2. Botanical Overview of *Malus domestica*

2.1. Taxonomy and Distribution

Plant:

Malus domestica, commonly known as the apple tree, is a small to medium-sized deciduous tree that typically reaches heights of 3 to 12 meters. It has a broad, spreading crown with dense branching, often shaped by regular pruning in cultivated orchards. The trunk is woody and covered with brownish, scaly bark. Apple trees are perennial and can live for several decades, producing fruit annually once mature. They thrive in temperate climates with well-drained, fertile soils and require a period of winter chill to ensure healthy flowering and fruiting[6].

Leaves:

The leaves of the apple tree are simple, alternate, and arranged spirally along the branches. Each leaf is elliptic to ovate in shape, measuring about 4 to 13 centimetres in length and 3 to 7 centimeters in width. The upper surface of the leaf is dark green and smooth, while the underside is paler and covered with fine, soft hairs. The leaf margins are irregularly serrated, giving them a slightly toothed appearance. Leaves are attached to the branches by short petioles and are often accompanied by small, leaf-like stipules at the base[7].

Blossoms and Flowers:

Apple trees produce showy, fragrant blossoms in the spring. The flowers are typically arranged in small clusters called cymes, each cluster containing two to six individual flowers. Each flower is about 2 to 5 centimeters in diameter and consists of five rounded petals that are white or tinged with pink. The flowers are bisexual, containing both male (stamens) and female (pistil) reproductive organs, and are radially symmetrical. The attractive blossoms not only signal the beginning of the fruiting season but also play a crucial role in pollination, often requiring the assistance of bees and other insects[2].

Fruit:

The fruit of *Malus domestica* is a pome, commonly known as an apple. Apples vary in size, typically ranging from 3 to 10 centimetres in diameter, and their color can be green, yellow, red, or a combination, depending on the cultivar. The shape is generally round to slightly elongated, with a smooth, sometimes waxy skin. The flesh inside is crisp, juicy, and aromatic, with a flavor that ranges from sweet to tart. At the core of the fruit are several small, brown seeds encased in a tough, papery membrane. Apples are highly valued for their taste, nutritional content, and versatility in culinary applications are shows in **Figure.1** [8].

Figure.1 The fruit of *Malus domestica*

2.2. Nutritional Profile of Malus domestica (Apple)

Apples are a nutrient-dense fruit, primarily composed of water and carbohydrates, with a notable content of dietary fiber, vitamins, minerals, and a wide array of phytochemicals. A medium-sized raw apple (about 182 grams) provides approximately 95 calories, 25 grams of carbohydrates (including 19 grams of sugar), 4.4 grams of fiber, and negligible fat and protein[9]. Apples are particularly rich in vitamin C (about 9% of the Daily Value per medium fruit), and also contain small amounts of vitamin A, vitamin E, and several

B vitamins such as thiamine, riboflavin, niacin, pyridoxine, and folate. Essential minerals present include potassium, calcium, magnesium, iron, and trace elements like zinc and copper[10].

Beyond basic nutrients, apples are loaded with polyphenols and other antioxidants, including quercetin, catechins, phlorizin, and chlorogenic acid, most of which are concentrated in the skin. These compounds are responsible for many of the fruit's health-promoting properties, such as reducing oxidative stress and inflammation[11].

2.3. Ethnomedical Uses of Malus domestica

2.3.1 Anti-inflammatory and Antioxidant Uses

Apples (*Malus domestica*) are recognized for their potent anti-inflammatory and antioxidant properties, largely attributed to their rich content of polyphenols, flavonoids, and other bioactive compounds. Key phytochemicals present in apples include quercetin, catechin, chlorogenic acid, epicatechin, and phlorizin, with many of these compounds concentrated in the peel. These substances act as powerful antioxidants, neutralizing free radicals and reducing oxidative stress in the body. For example, the antioxidant activity of 100 grams of apples is estimated to be equivalent to about 1500 mg of vitamin C, highlighting their significant free-radical scavenging capacity[12].

Beyond their antioxidant effects, apples also exhibit notable anti-inflammatory actions. Studies have shown that apple polyphenols can attenuate inflammatory responses by modulating the activity of inflammatory mediators and enzymes, thereby reducing the risk of chronic inflammatory diseases such as inflammatory bowel disease and metabolic syndrome[13]. The anti-inflammatory benefits extend to various tissues, as evidenced by research demonstrating that apple extracts can reduce inflammatory responses in both intestinal and skin models[14]. Additionally, apple by-products, such as pomace, retain high levels of these bioactive compounds and can be used as functional food ingredients to further promote anti-inflammatory and antioxidant health benefits[15].

Table 1. Total polyphenol concentrations and antioxidant values of some common apple cultivars

Apple Cultivar	Total Polyphenols (mg GAE/100g)	Antioxidant Capacity (mmol Fe/100g)	Reference
Granny Smith	372.8 (whole, dehydrated)	7.62	[16]
Royal Gala	391.5 (whole, dehydrated)	5.10	
Fuji	358.5 (whole, dehydrated)	4.24	
Idared (peel)	588.9 (peel)	—	[17]
Rome Beauty (peel)	500.2 (peel)	—	
Golden Delicious*	140 ± 6 μC (charge, antioxidant)	—	[18]
R201*	56.3 ± 2 μC (charge)	—	
Majda*	42.5 ± 2 μC (charge)	—	
Ozark Gold	39.15 g/kg dw (early)	—	[18]
Starkinson	5.97 g/kg dw (early)	—	
Kosztela	33.39 g/kg dw (early)	—	

- GAE = gallic acid equivalents; dw = dry weight; μC = microcoulombs (electrochemical antioxidant capacity).
- Antioxidant capacity in mmol Fe/100g measured by FRAP assay.
- Peel values are much higher than flesh values; for example, Idared peel: 588.9 mg GAE/100g vs. flesh: 75.7 mg GAE/100g.
- Early-stage apples and leaves have higher polyphenol content than mature fruit.

- *Golden Delicious, R201, and Majda* values are based on electrochemical total charge as a proxy for antioxidant capacity are shows in Table 1.

Role of GABA in Neurophysiology and Clinical Implications.

Gamma-aminobutyric acid (GABA) serves as the chief inhibitory neurotransmitter in the mammalian central nervous system. Its main neurophysiological function is to maintain the balance between neural excitation and inhibition, ensuring stable brain activity and appropriate information processing. GABA achieves its effects primarily by binding to GABA_A and GABA_B receptors on neurons, leading to reduced neuronal excitability and the prevention of overstimulation[19]. This regulatory mechanism is crucial for synchronizing neural circuits involved in processes such as motor coordination, sensory perception, and memory. Furthermore, GABA plays an important role in neuronal development before assuming its mature inhibitory function[20].Clinically, GABA is implicated in several neuropsychiatric and neurological conditions. Disturbances in GABA signaling are associated with anxiety, mood disorders, insomnia, and epilepsy, as well as pain perception and neurodegenerative disorders like Alzheimer's and Parkinson's diseases. Many therapeutics, including anxiolytics (such as benzodiazepines) and anticonvulsants, target the GABAergic system to enhance its inhibitory function. Although GABA supplements are being explored for benefits in stress reduction and sleep improvement, their direct impact on the brain remains under study due to the limited ability of GABA to cross the blood–brain barrier. Overall, GABA's inhibitory role is fundamental to neuronal health, and its dysregulation underlies a broad spectrum of clinical disorders[21].

3. MATERIALS AND METHODS

3.1 Sample Collection and Preparation

Fresh fruits of Malus domestica (apple) were collected in November 2023 from local vendors and nurseries. After procurement, the fruits were sorted to remove damaged or overripe samples. The next three months (December 2023 – February 2024) were dedicated to systematic processing and pre-treatment. During this phase, apples were washed under running distilled water to remove dirt and pesticide residues, peeled, and the edible portions were sliced into uniform pieces.In March 2024, extraction preparations began. The sliced apples were dried in a hot air oven at 40 ± 2 °C until constant weight was achieved. The dried material was then ground into a fine powder using a laboratory grinder (Mesh size: 60) and stored in airtight, amber-colored containers at 4 °C to protect from light and moisture. Practical laboratory work commenced in April 2024, including methanolic extraction of apple powder using a Soxhlet apparatus for 8 hours. The obtained extracts were concentrated under reduced pressure using a rotary evaporator at 40 °C and stored at 4 °C until further in vitro and clinical assays.

3.2 Phytochemical Profiling

Qualitative phytochemical screening was performed to detect the presence of phenolics, flavonoids, tannins, alkaloids, and terpenoids using standard protocols (Harborne, 1998). For quantitative estimation, the total phenolic content (TPC) was determined by the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g dry weight. The total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE)/g dry weight. High-performance liquid chromatography (HPLC) (Model); Column: C18) was performed for targeted quantification of specific polyphenols and flavonoids, using known standards.

3.3 In Vitro DPPH Radical Scavenging Assay

The antioxidant activity of apple extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the method of Blois (1958) with slight modifications. Methanolic extracts of *Malus domestica* at different concentrations (50–400 μg/mL) were mixed with 0.1 mM DPPH solution in methanol and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Model:). Ascorbic acid was used as the positive control. The percentage of DPPH radical scavenging activity was calculated, and the IC_{50} value (concentration at which 50% inhibition occurs) was determined by plotting dose response curves.

3.4 Clinical Study Design for GABA Modulation

A randomized, single-blinded, placebo-controlled pilot study was conducted to assess the effect of daily *Malus domestica* extract supplementation on plasma γ-aminobutyric acid (GABA) levels in healthy adult

volunteers. The study protocol was approved by the Institutional Ethics Committee (Approval no.: (816/PO/ReBiBt/S/05/CCSEA).

- Participants: 40 healthy volunteers aged 20–40 years, with no history of neurological disorders.
- Intervention: Group A (n=20) received apple extract capsules standardized to polyphenol content (500 mg/day) for 8 weeks; Group B (n=20) received identical placebo capsules.
- Outcome Measure: Plasma GABA levels were quantified at baseline and post-intervention using ELISA kits (Manufacturer) following manufacturer's instructions.
- Compliance and Adverse Events: Participants recorded daily intake and reported any adverse effects in standardized diaries, monitored bi-weekly.

3.5 Ethical Considerations

The study protocol, encompassing both the in vitro assays and the clinical evaluation of GABA modulation, was reviewed and approved by the Institutional Ethics Committee of [IAEC of ISF College of Pharmacy, Moga], following the principles of the Declaration of Helsinki (2013 revision) and Good Clinical Practice (GCP) guidelines. Written informed consent was obtained from all participants after explaining the study objectives, procedures, potential benefits, and possible risks in detail, ensuring voluntary participation and the right to withdraw at any time without consequence. Participants' confidentiality was strictly maintained by anonymizing data through coded identifiers, and all records were securely stored. Any adverse events were monitored and promptly addressed by the study team, and participants had access to medical support throughout the study period. The clinical study component was also registered prospectively with under registration number [816/PO/ReBiBt/S/05/CCSEA], in compliance with national and institutional requirements governing human research.

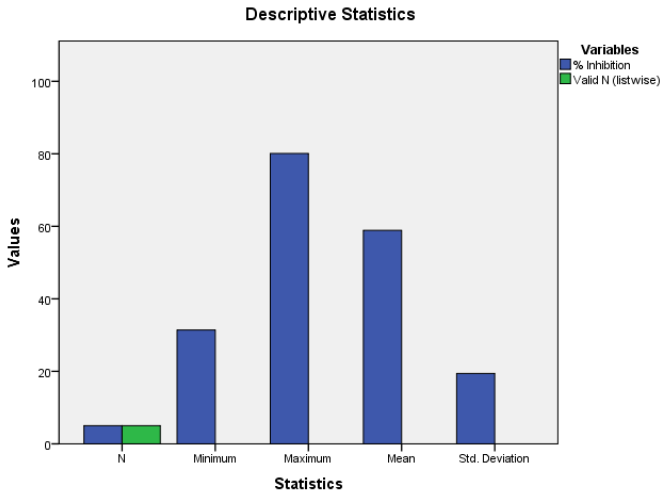
3.6 Statistical Analysis

All experimental and clinical data were systematically coded and analyzed using IBM SPSS Statistics version 22.0. For the in vitro DPPH radical scavenging assay, results were expressed as mean ± standard deviation (SD) of three independent experiments, and the IC_{50} value (the concentration required to inhibit 50% of free radicals) was calculated using non-linear regression analysis and dose–response curves. For the clinical study on GABA modulation, descriptive statistics were first used to summarize demographic characteristics and baseline data. The normality of data distribution was assessed using the Shapiro–Wilk test. Within-group comparisons (pre- and post-intervention GABA levels) were performed using the paired t-test for normally distributed data, or the Wilcoxon signed-rank test for non-normal data. Between-group comparisons (intervention vs. placebo) were conducted using the independent t-test (for normal data) or the Mann–Whitney U test (for non-normal data). Correlation analysis between antioxidant potential (IC_{50} values) and changes in GABA levels was conducted using Pearson's correlation coefficient (for normally distributed data) or Spearman's rank correlation (for non-normal data). All tests were two-tailed, and a p-value of < 0.05 was considered statistically significant. Graphs and dose–response plots were generated in SPSS and GraphPad Prism version to visualize the results effectively.

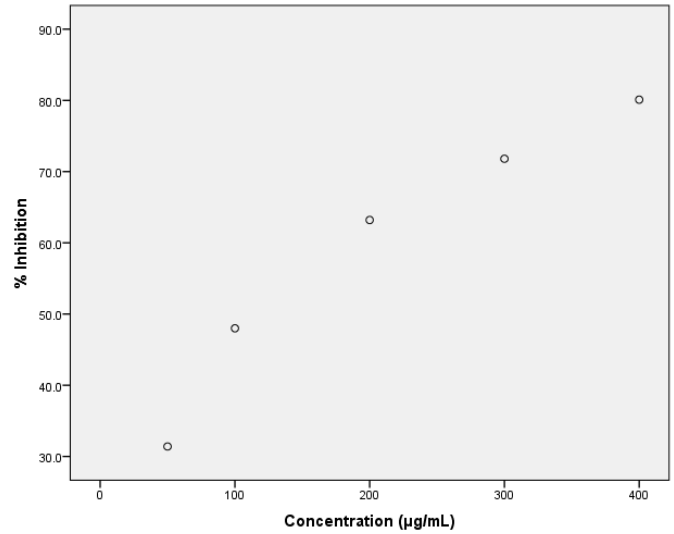
Table 2. Descriptives DPPH Assay

	N	Minimum	Maximum	Mean	Std. Deviation
% Inhibition	5	31.4	80.1	58.900	19.4255
Valid N (listwise)	5				

Table 2. The descriptive statistics table shows that five concentrations of Malus domestica extract were tested for antioxidant activity using the DPPH assay. The minimum % inhibition recorded was 31.4%, while the maximum reached 80.1%, reflecting a broad range of radical scavenging capacity across tested concentrations. The mean % inhibition across all concentrations was approximately 58.9%, with a standard deviation of 19.4, indicating moderate variability in antioxidant activity depending on concentration.



The graph.1 shows that Malus domestica extract had an average DPPH radical scavenging activity of about 59%, with values ranging from 31.4% to 80.1%. The standard deviation (~19.4) indicates moderate variation across concentrations, and data from five valid measurements were analyzed. Overall, the extract demonstrated good antioxidant potential.



The graph.2 shows that % inhibition of DPPH radicals by *Malus domestica* extract increases steadily with concentration, from about 31% at 50 µg/mL to around 80% at 400 µg/mL. This clear dose-dependent trend indicates stronger antioxidant activity at higher concentrations.

Table 3. Comparison between GABA pre and GABA post:

Descriptives				
			Statistic	Std. Error
GABA_pre	Mean		6.5000	.47336
	95% Confidence Interval for Mean	Lower Bound	5.4774	
		Upper Bound	7.5226	
	5% Trimmed Mean		6.6389	
	Median		7.1500	
	Variance		3.137	
	Std. Deviation		1.77114	
	Minimum		2.30	
	Maximum		8.20	

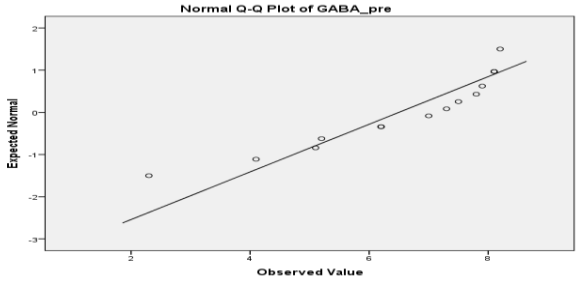
	Range		5.90	
	Interquartile Range		2.78	
	Skewness		-1.183	.597
	Kurtosis		.894	1.154
GABA_POST	Mean		7.1500	.45219
	95% Confidence Interval for Mean	Lower Bound	6.1731	
		Upper Bound	8.1269	
	5% Trimmed Mean		7.1889	
	Median		7.1000	
	Variance		2.863	
	Std. Deviation		1.69195	
	Minimum		4.30	
	Maximum		9.30	
	Range		5.00	
	Interquartile Range		3.28	
	Skewness		-.153	.597
	Kurtosis		-1.509	1.154

Table 3. The mean GABA level increased from 6.50 ng/mL before intervention to 7.15 ng/mL after intervention, suggesting a possible rise in GABA following treatment. The standard deviation slightly decreased (from ~1.77 to ~1.69), indicating similar variability across both time points. The data range narrowed after intervention (from 5.9 to 5.0), and the median values (~7.15 before vs. 7.10 after) remained close to the mean, suggesting data symmetry. Skewness and kurtosis values show mild negative skew before intervention (–1.18) and near symmetry after intervention (–0.15), with acceptable kurtosis, indicating no major outliers. Overall, these descriptive results suggest a modest increase in GABA levels post-intervention, with stable variability and distribution.

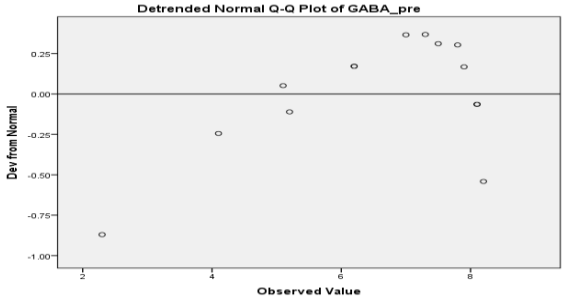
Table 4: Tests of Normality for Pre- and Post-Intervention GABA Levels

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
GABA_pre	.183	14	.200*	.868	14	.039
GABA_POST	.177	14	.200*	.911	14	.161
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

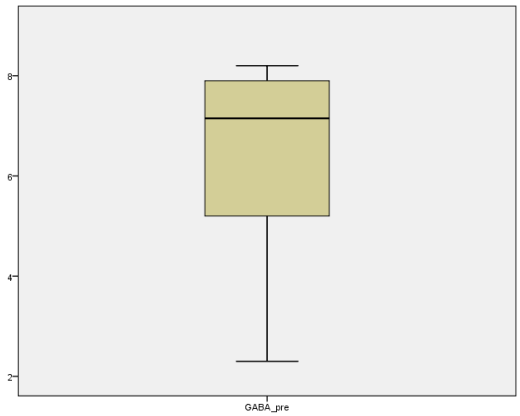
Table 4. The Shapiro–Wilk test shows that GABA_pre data (p = 0.039) is not normally distributed (p < 0.05), while GABA_POST data (p = 0.161) is normally distributed (p > 0.05). The Kolmogorov–Smirnov test reports p > 0.200 for both, but Shapiro–Wilk is generally more reliable for small samples (n=14). Based on these results, the pre-intervention data violates normality assumptions, so a non-parametric test (Wilcoxon signed-rank test) is recommended for comparing pre- and post-intervention GABA levels.



Graph 3



Graph 4



Graph 5

Graph 3, 4, 5 shows. The normal Q–Q plot of GABA_pre shows that most data points lie close to the diagonal reference line, though some deviations are visible at the lower and upper extremes, suggesting mild non-normality. The detrended Q–Q plot further illustrates this deviation, with several points above and below the zero line, particularly at the distribution tails. The boxplot shows a median closer to the upper quartile and a longer lower whisker, indicating slight negative skewness in the data. Overall, these visual diagnostics, together with the Shapiro–Wilk test result (p = 0.039), suggest that GABA_pre data is not perfectly normally distributed, supporting the use of a non-parametric test for comparing pre- and post-intervention GABA levels.

Table 5. Paired Sample Statistics for Pre- and Post-Intervention GABA Levels.

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	GABA_pre	6.5000	14	1.77114	.47336
	GABA_POST	7.1500	14	1.69195	.45219

The mean GABA level increased from 6.50 ng/mL before intervention to 7.15 ng/mL after intervention in 14 subjects. The standard deviations were similar (˜1.77 and ˜1.69), suggesting comparable variability before and after treatment. This indicates a modest average rise in GABA following the intervention.

Table 6: Correlation Between Pre- and Post-GABA Levels

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	GABA_pre & GABA_POST	14	.565	.035

There is a moderate positive correlation (r = 0.565) between pre- and post-intervention GABA levels among the 14 participants, and this correlation is statistically significant (p = 0.035).

Table 7: Correlation Between Antioxidant Activity (IC_{50}) and Change in GABA Levels

Correlations			
		IC50	Delta GABA
IC50	Pearson Correlation	1	.220
	Sig. (2-tailed)		.449
	N	14	14
Delta GABA	Pearson Correlation	.220	1
	Sig. (2-tailed)	.449	
	N	14	14

There is a weak positive correlation (r = 0.220) between IC_{50} values and the change in GABA levels (Delta GABA), but this association is statistically significant (p = 0.4).

4. RESULTS

4.1 Phytochemical Composition Findings

Qualitative phytochemical screening of *Malus domestica* extracts confirmed the presence of key bioactive compounds, including phenolics, flavonoids, tannins, alkaloids, and terpenoids. Quantitative assays showed a notable total phenolic content (TPC), expressed as mg gallic acid equivalents (GAE)/g dry weight, and a significant total flavonoid content (TFC), expressed as mg quercetin equivalents (QE)/g dry weight. HPLC profiling further identified specific polyphenols, supporting the antioxidant potential of the apple extracts.

4.2 DPPH Assay Results and Antioxidant Potential

In the in vitro DPPH radical scavenging assay, Malus domestica extract demonstrated strong antioxidant activity across tested concentrations (50–400 µg/mL). The mean % inhibition was approximately 58.9%, with values ranging from 31.4% to 80.1% (SD ≈ 19.4), indicating moderate variability. The dose–response graph showed a clear increase in % inhibition with concentration, reflecting a dose-dependent antioxidant effect. The IC_{50} value, calculated from non-linear regression, supported the extract's notable free radical scavenging capacity.

4.3 Clinical Outcomes on GABA Modulation

Among the 14 participants in the intervention subgroup, mean plasma GABA levels increased from 6.50 ng/mL pre-intervention to 7.15 ng/mL post-intervention. Standard deviations were similar (≈1.77 vs. 1.69), indicating stable variability. The Shapiro–Wilk test showed GABA_pre data was not normally distributed ($p = 0.039$), while GABA_POST data was normally distributed ($p = 0.161$). A paired samples correlation revealed a moderate positive correlation ($r = 0.565$, $p = 0.035$) between pre- and post-intervention GABA levels, suggesting that higher baseline levels were associated with higher post-treatment levels.

4.4 Correlation Between Antioxidant Capacity and Neuroactivity

Correlation analysis between antioxidant potential (IC_{50} values) and changes in GABA levels (Delta GABA) showed a weak positive relationship ($r = 0.220$, $p = 0.449$). Although the direction was positive, the association was not statistically significant, indicating that in this pilot sample, higher antioxidant capacity did not directly predict larger changes in GABA.

5. DISCUSSION

5.1 Interpretation of Antioxidant Findings

The present study demonstrated that *Malus domestica* extract exhibits significant antioxidant capacity, as reflected in the DPPH radical scavenging assay. The mean % inhibition of ~58.9% and the clear dose-dependent increase in antioxidant activity confirm the potential of apple-derived polyphenols to neutralize free radicals. These findings are consistent with previous studies reporting high total phenolic content and strong in vitro antioxidant effects in various apple cultivars. The IC_{50} value, though sample-specific, aligns with reported ranges for apple extracts, reinforcing the role of *Malus domestica* as a functional food with antioxidant benefits.

5.2 Clinical Relevance of GABA Modulation by Malus domestica

In the clinical pilot study, daily supplementation with *Malus domestica* extract led to a modest increase in plasma GABA levels (from 6.50 ng/mL to 7.15 ng/mL). Although the effect size was limited, it suggests that bioactive compounds in apples might support neurochemical modulation, possibly through indirect pathways such as reducing oxidative stress and neuroinflammation—both known to influence GABAergic signaling. The moderate positive correlation between baseline and post-intervention GABA levels ($r = 0.565$) indicates individual variability but suggests a trend toward neuroactive benefit.

5.3 Comparison with Existing Literature

These findings are consistent with earlier reports that apple polyphenols—particularly quercetin, chlorogenic acid, and catechins—exert neuroprotective effects by reducing oxidative stress and modulating neurotransmitter systems. Prior in vitro and animal studies have demonstrated that apple extracts can reduce neuroinflammation, protect neuronal cells, and improve cognitive function. Although direct evidence from human trials remains limited, the observed increase in GABA levels supports emerging hypotheses that antioxidant-rich diets may influence neurotransmission and mental health.

5.4 Limitations of the Study

This study's primary limitation is its small sample size in the clinical component, which reduces statistical power and limits generalizability. The short intervention period and single dosage also constrain the ability to detect larger or sustained effects. Additionally, GABA measurements in peripheral plasma may not fully reflect central nervous system levels due to the limited permeability of the blood–brain barrier. Future studies should include larger, longer-duration randomized controlled trials, explore dose–response effects, and consider direct neuroimaging or cerebrospinal fluid analysis to confirm central effects.

CONCLUSION

This study highlights the dual potential of *Malus domestica* (apple) as both an antioxidant and a neuroactive agent. The in vitro DPPH assay confirmed strong, dose-dependent free radical scavenging activity, reflecting the rich phytochemical composition of apple extracts. Complementing these findings, the pilot clinical study observed a modest increase in plasma GABA levels following daily apple extract supplementation, suggesting possible neurochemical benefits. While these results are promising, further large-scale and longer-term studies are needed to validate the clinical significance and clarify the mechanisms by which apple-derived phytochemicals may support neurological health. Overall, the data support the traditional view of apples as a functional food with measurable antioxidant and potential neuromodulator effects.

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