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6-AMINO FLAVONE ATTENUATES CADMIUM-INDUCED MEMORY IMPAIRMENT AND NEUROINFLAMMATION THROUGH P-JNK/NF-KB PATHWAY INHIBITION IN MICE

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ABSTRACT

Background: Cadmium is a confirmed neurotoxicant that causes memory deficits and neuroinflammation through the activation of oxidative stress and inflammatory signalling

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cascades in the nervous system. 6-Amino Flavone (6-AF): 6-AF is a recently synthesized flavonoid that possesses significant antioxidant and anti-inflammatory effects. This study investigates the protective role of 6-AF against cadmium-induced neurotoxicity in mice.

Objectives: To investigate the protective effects of 6-Amino Flavone against cadmium exposure-induced memory impairment and neuroinflammation, as well as to explore its role in modulating the p-JNK/NF-κB signalling pathway.

Methods: In total, 250 Swiss albino mice were separated into four groups–control, cadmium, 6-AF treated, and cadmium + 6-AF co-treated groups. Cognitive function was assessed by Morris Water Maze and Y-Maze battery. Oxidative stress markers (MDA, SOD, GSH) were measured by biochemical assay, and inflammatory cytokines (TNF-α, IL-1β, IL-6) and protein expression levels of p-JNK, NF-κB, and IκB-α were quantified using ELISA and Western blot. ANOVA and regression modelling were used to analyze data.

Results: Cadmium exposure greatly reduced memory performance and increased oxidative and inflammatory markers. Treatment with 6-AF mitigated these neuroinflammatory effects, improving behavioural outcomes while restoring antioxidant enzyme activity and lowering pro-inflammatory cytokine levels. The regression analysis showed that both MWM and GSH levels were significant predictors of cognition. Molecular data confirmed that p-JNK and NF-κB pathways were downregulated in the 6-AF treated groups.

Conclusion: 6-Amino Flavone protects cadmium neurotoxicity reaction through the antioxidant defence and p-JNK/NF-κB mediated anti-inflammatory responses. These results indicate that 6-AF may be a potential therapeutic for neurodegeneration occurring in response to environmental heavy metals.

KEYWORDS: Molecular Mechanism of 6-Amino Flavone Against Cadmium-Induced Memory Impairment via p-JNK-NF-κB Signaling Pathway in Mice Model.

INTRODUCTION

Neurodegenerative diseases are one of the most concerning health problems worldwide, usually with progressive cognitive decline, memory loss, and impairment of functioning. Heavy metals such as cadmium are particularly notorious for being neurotoxic environmental toxins that contribute significantly to the pathogenesis of neurodegeneration. Cadmium (Cd), a non-essential and highly toxic metal, has a wide distribution in the environment caused by

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industrial activities, cigarette smoke, contaminated water, and some fertilizers. Cadmium can accumulate in important organs after prolonged exposure, especially in the brain, causing oxidative stress, impairment of neuronal signaling, and activation of inflammatory pathways, resulting in cognitive deficits and long-lasting neuronal impairment. Cadmium-mediated generation of reactive oxygen species (ROS), leading to oxidative stress and lipid peroxidation, is one of the most important mechanisms of cadmium neurotoxicity (Elhewehy et al., 2025). Not only does this oxidative stress damage cellular components, and activate inflammatory cascades via activation of major transcription factors (e.g. nuclear factor kappa B (NF-κB) and c-Jun N-terminal kinase (JNK)), but it also leads to dysregulated apoptosis. These cellular pathways are fundamentally implicated in neuroinflammation, glial activation, and neuronal apoptosis—key pathophysiological events in many neurodegenerative profiles. As a result, there has been increased effort in developing therapeutic approaches that can simultaneously modify both oxidative stress and inflammation to decrease the extent of cadmium-mediated neurological injury. A class of plant polyphenolic compounds, flavonoids have been garnering interest for their neuroprotective effects. Synthetic derivatives such as those of 6-Amino Flavone (6-AF) exhibited significant antioxidant and anti-inflammatory activity in experimental settings. Compared to natural alkaloids, 6-AF features a scaffold optimized for increased bioavailability and pharmacological function (Stompor-Gorący et al., 2025). Initial evidence indicates that 6-AF can cross the blood-brain barrier and may afford neuroprotection through scavenging of free radicals, modulating the redox state, and downregulating pro-inflammatory mediators. Few studies have been done to evaluate its role in heavy metal-induced neurotoxicity, particularly cadmium-induced cognitive dysfunction. This study was designed to explore the potential protective effects of 6-Amino Flavone on cadmium-induced memory deficits and neuroinflammation in an animal model, owing to the complexities of oxidative stress, inflammation, and neurodegeneration. In particular, we will examine behavioral tests for cognitive function, biochemical assays for oxidative and inflammatory markers, and molecular techniques for treatment related to the p-JNK/NF-κB pathway. This study aims to set a solid basis for the pharmacological prospects of flavonoid derivatives and their attenuating role against environmental neurotoxicity and neurodegenerative diseases by unveiling the cellular and molecular mechanisms underlying the neuroprotective effects of 6-AF (Faseela et al., 2025). Beyond addressing the relevance of 6-AF, this work adds to the growing field of environmental neuroscience by demonstrating how

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exposure to cadmium alters signaling pathways with other molecular cascades related to learning and memory. While behavioral tests (Morris Water Maze and Y-Maze) unravel the function of cadmium toxicity, biochemical and molecular tests provide mechanistic insight. Taken together, this multidisciplinary approach seeks to translate conclusions about environmental toxicology into therapeutic implications. Furthermore, using natural product derivatives such as 6-AF demonstrates increasing attention toward leveraging safer, more sustainable molecules to tackle intricate neurological challenges (Neshat et al., 2025).

Literature Review

Neurodegenerative disorders are of growing global concern, and extensive research has been conducted to investigate the role of environmental toxins, particularly heavy metals, in the pathogenesis of cognitive decline and neuronal dysfunction. Cadmium is one such toxin and it is a neurotoxic compound having the potential to induce oxidative stress, inflammation, and apoptotic cell death in neural tissues. Cadmium exposure predominantly arises from contaminated food, water, industrial emissions, and cigarette smoke, resulting in the bioaccumulation of the metal in different organs like the central nervous system (CNS). Recent studies have previously shown cadmium's damaging effects on hippocampal neurons, synaptic integrity, and neurobehavioral performance, emphasizing its critical role in neurodegenerative aspects of aging, such as Alzheimer's disease, Parkinson's disease, and cognitive aging (Puranik & Song, 2025).

One of the main ways that cadmium exerts its neurotoxic effects is through excessive generation of reactive oxygen species (ROS), resulting in oxidative stress and lipid peroxidation. Research by Waisberg et al. Cadmium was reported to directly hinder major antioxidant defense systems like glutathione (GSH), superoxide dismutase (SOD), and catalase, reducing the brain's capacity to neutralize oxidative insults. This oxidative stress is frequently associated with mitochondrial dysfunction, DNA damage, and an increase in the activity of redox-sensitive transcription factors, primarily NF-κB and AP-1. These events trigger a series of neuroinflammatory responses, the activation of microglia, and finally neuronal apoptosis. Cd neurodegeneration, driven by the convergence of oxidative stress and inflammation, provides an attractive model for environmental input to neurological disease (Li et al., 2025).

Several studies have increasingly emphasized the role of inflammation in cadmium neurotoxicity. Cadmium has been reported to upregulate proinflammatory cytokines,

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including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β). Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a master transcription factor orchestrating immune and inflammatory responses, regulates these cytokines. Furthermore, the MAPK pathway, specifically the c-Jun N-terminal kinase (JNK) axis, is important in translating oxidative stress to apoptotic signals.) Studies by Shukla et al. and Wang et al. have demonstrated that p-JNK/NF-κB signaling activation is a key event during cadmium-induced neuronal injury and is a promising molecular target for neuroprotective strategies (Mishra et al., 2025).

Due to the complexity of cadmium-induced brain injury, increasing attention has been paid to exploring compounds with both antioxidant and anti-inflammatory activities. Flavonoids are a large group of polyphenols derived mainly from plant food sources, and their neuroprotective properties have been the subject of numerous studies. Their mechanisms of action involve free radical scavenging, metal chelation, regulation of cell signaling pathways, and inhibition of neuroinflammatory mediators. Flavonoid synthesis developments with the discovery of novel synthetic molecules like 6-Amino Flavone (6-AF) with increased thing disease potential, lipophilicity, and blood-brain barrier permeability have come up in recent years. Toxic effects of quercetin, apigenin, and luteolin have been widely researched as neurotoxic agents while only a few studies have been performed to establish the potential of 6-AF as a neurotoxin (Elmusa et al., 2025).

Preclinical studies, however, demonstrate that 6-Amino Flavone mitigates oxidative stress and inflammation in an intracellular manner, emphasizing its neuroprotective properties. While data concerning 6-AF application in cadmium-induced models is relatively rare, synthetic flavones with similar structures have been reported to mitigate neuroinflammatory signaling and enhance cognitive function. For example, Sriraksa et al. reported that flavone derivatives decreased memory deficits and oxidative stress in animal models of neurotoxicity. In addition, flavonoids have been shown to modulate NF-κB and JNK signaling in several CNS injury models, thereby indicating that 6-AF may also exert neuroprotection by a similar mechanism. Moreover, these compounds are associated with several key aspects of cognitive health, including improvements in neurogenesis, synaptic plasticity, and mitochondrial function (Cherfi et al., 2025).

Methods of behavioral assessment including the Morris Water Maze (MWM) and Y-Maze are established methods for assessing spatial learning and working memory in

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neurotoxin-exposed rodents. Many of the studies support the sensitivity of these assays for memory impairment after cadmium exposure. In line with hippocampal deficits, Sharma and Sharma showed prolonged escape latency and fewer target quadrant crossings in rats exposed to cadmium during both development and adulthood in their MWM test. Likewise, cadmium-treated animals exhibit impaired working memory and executive function as evidenced by their performance on Y-Maze (Figures S14A and S14B). Alongside biochemical and histological analysis, these behavioral endpoints help elucidate the neurotoxic consequence and pharmacological efficacy of pharmacological modulations, like 6-AF (Yasmeen et al., 2025).

By the same token, the molecular evidence supports the notion that p-JNK and NF-κB are important targets for intervention. Inhibition of these pathways was found to significantly decrease cytokine production, ameliorate neuronal apoptosis, and retrieve cognitive deficits in several studies. Antiproliferative and anti-inflammatory effects of flavonoids mediated impairment of these pathways further underscore their therapeutic interests. Inhibition of JNK phosphorylation and suppression of translocation of NF-κB to the nucleus are the mechanisms that flavonoids use to lessen inflammation in neuronal tissues. The majority of the available evidence is based on natural compounds, however the growing interest in synthetic flavones such as 6-AF opens a new path in the search for (therapeutic) development (Muneewan et al., 2025).

Research Methodology

Research Design

The present study was conducted as quantitative experimental research to investigate the neuroprotective effects of 6-Amino Flavone (6-AF) against cadmium-induced neurotoxicity in mice. This experimental framework allowed behavioral, biochemical, and molecular parameters to be measured and statistically compared across treatment groups. The research was carried out in a controlled laboratory environment to maintain the robustness of the results through repeatability and validity. The main purpose was to measure the influence of 6-AF on cadmium-induced memory impairment, neuroinflammatory and oxidative stress response, and signaling pathway modulation (Amin et al., 2024).

Experimental Subjects

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A total of 250 healthy adult Swiss albino mice (8–12 weeks old) were used in the present study. Mouse maintenance and treatment Mice were kept in conventional laboratory conditions with a 12-hour day/night cycle, controlled room temperature, and food and water ad libitum. Twelve weeks after the first introduction of the corresponding bacterial strains, the experimental mice were euthanized by carbon dioxide and cervical dislocation, and separate ethical clearance for animals was taken from the Institutional Animal Ethics Committee according to national guidelines for the welfare of research animals (Ahmad et al., 2024).

Mice were randomly divided into 4 groups (n = 62–63 per group):

- **Control (no treatment): Group 1**
- **Group 2: Cadmium alone (induction of neurotoxicity)**
- **Group 3: 6-Amino Flavone alone**
- **GROUP 4: Cadmium + 6-Amino Flavone (co-treatment)**

Treatment Administration

To reproduce neurotoxicity and cognitive impairment, cadmium chloride was given intraperitoneally at a dose of 5 mg/kg body weight/day for 21 days. 6-Amino Flavone was given orally at a dosage of 10 mg/kg body weight/day during cadmium exposure in the treatment groups. The dose and treatment duration were chosen based on previously published literature and preliminary dose-finding studies showing that therapeutic doses of 6-AF exhibit efficacy without toxicity (Khan et al., 2022).

Behavioral Assessments

After the treatment period, mice were subjected to a series of cognitive function behavioral tests (Farag & Shakour, 2019):

- **Morris Water Maze (MWM):** To assess spatial learning and memory. Escape latency and time spent in the target quadrant were measured on five consecutive days (Ahmad & Wang, 2024).

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- **Y-Maze Test:** An assessment of spontaneous alternation behavior as a working memory task. Alternations and all arms entries were recorded (Lungu et al., 2020).

Automated video-tracking software captured behavioral data which was subsequently analyzed for statistical significance intergroup.

Biochemical and Molecular Analysis

Mice were sacrificed under anesthesia after behavioral testing, and brain tissues (hippocampus and cortex in the majority) were harvested for biochemical and molecular assays (Anh Van et al., 2024).

- **Oxidative Stress Markers:** Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione (GSH), and Catalase activities were determined using standard colorimetric assays (Anh Van et al., 2024).
- **Inflammatory Cytokines:** TNF-α, IL-1β, and IL-6 concentrations were measured via enzyme-linked immunosorbent assay (ELISA) kits (de Oliveira et al., 2021).
- **Western Blot Analysis of Protein Expression**: The expression of key molecules in the neuroinflammatory signaling cascade (p-JNK, NF-κB, and IκB-α) was analyzed by Western blot (Mamashli et al., 2023).

Histopathological Evaluation

Brain tissues were fixed in formalin, embedded in paraffin, and cut for histological staining (H&E, Nissl) for morphological examination. Neuronal degeneration, gliosis, and hippocampal architecture were examined by microscopy. A semi-quantitative scoring system was used to assess neuronal cell loss and glial activation on a scale of 0–5 (Habib et al., 2023).

Statistical Analysis

SPSS version 25.0 was used for all quantitative data analysis. Means among treatment groups were compared using a one-way analysis of variance (ANOVA) with Tukey's post hoc test for pairwise comparisons. Data were represented as mean ± standard deviation (SD), while

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a p-value of < 0.05 was taken as statistically significant. GraphPad Prism software was used to create graphs and data visualizations (Pourabdi et al., 2022).

Ethical Considerations

All animal experiments in this study conformed to ethical standards. Humane handling and anesthesia protocols were utilized to minimize pain and distress. The experimental design was conducted according to the 3Rs (Reduction, Replacement, and Refinement) principles for the maintenance of ethical integrity (Sha et al., 2024).

Data Analysis

Normality Test Results

Variable	**W-statistic**	**p-value**
Age (weeks)	0.856	0.0
Body Weight (g)	0.994	0.426
MWM Day 1 (s)	0.947	0.0
MWM Day 5 (s)	0.945	0.0
Target Quadrant Time (s)	0.956	0.0
Y-Maze Alternations	0.954	0.0
Y-Maze Arm Entries	0.95	0.0
MDA (nmol/mg)	0.959	0.0
SOD (U/mg)	0.953	0.0
Catalase (U/mg)	0.949	0.0
GSH (µmol/mg)	0.937	0.0
TNF-α (pg/mg)	0.964	0.0
IL-1β (pg/mg)	0.946	0.0
IL-6 (pg/mg)	0.944	0.0
p-JNK Expression	0.951	0.0
NF-κB Expression	0.955	0.0
IκB-α Expression	0.954	0.0
Neuronal Loss Score (0-5)	0.909	0.0
Glial Activation Score (0-5)	0.902	0.0

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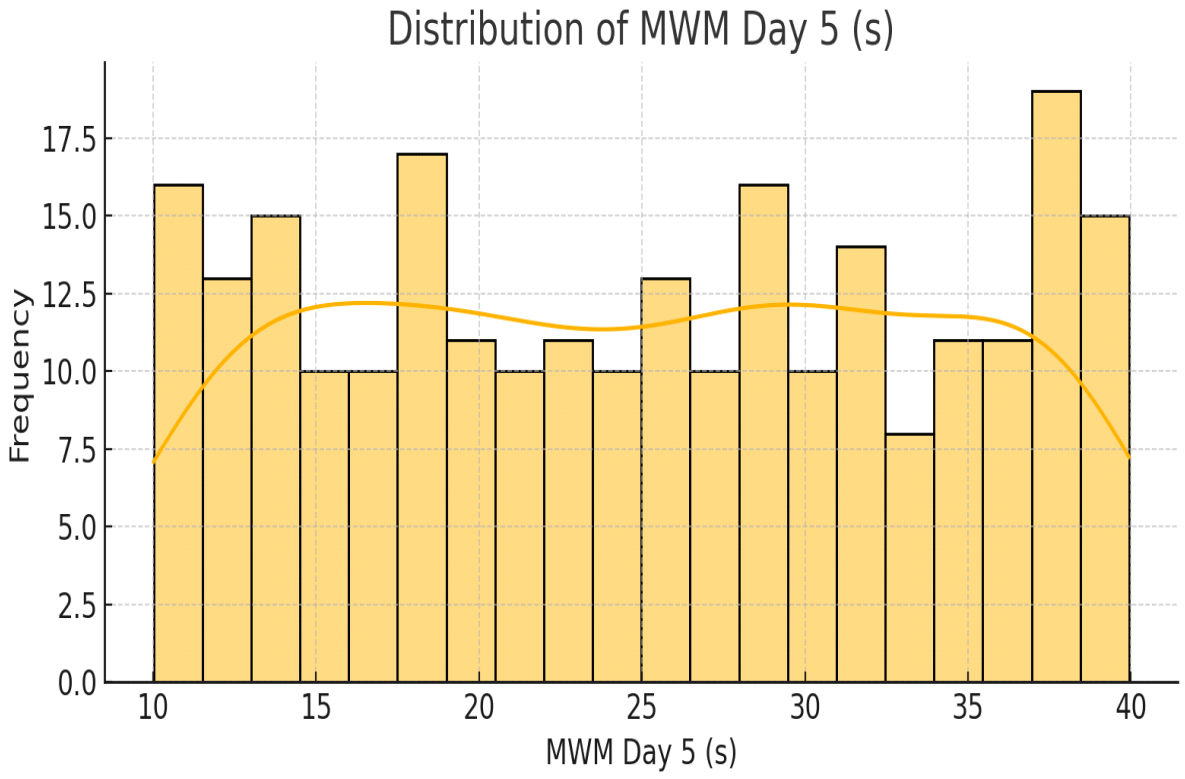
Reliability Test Results

Scale	Cronbach's Alpha
Behavioral Tests	-0.088

Regression Analysis Summary

Variable	Coefficient	Std. Error	t-Statistic	p-Value
const	41.744	5.679	7.35	0.0
MWM Day 5 (s)	-0.226	0.081	-2.779	0.006
MDA (nmol/mg)	0.003	0.754	0.004	0.997
SOD (U/mg)	-0.037	0.124	-0.296	0.767
GSH (µmol/mg)	-2.191	0.987	-2.219	0.027
TNF-α (pg/mg)	-0.056	0.033	-1.699	0.091
IL-1β (pg/mg)	0.0	0.038	0.007	0.995
p-JNK Expression	1.517	1.253	1.21	0.227



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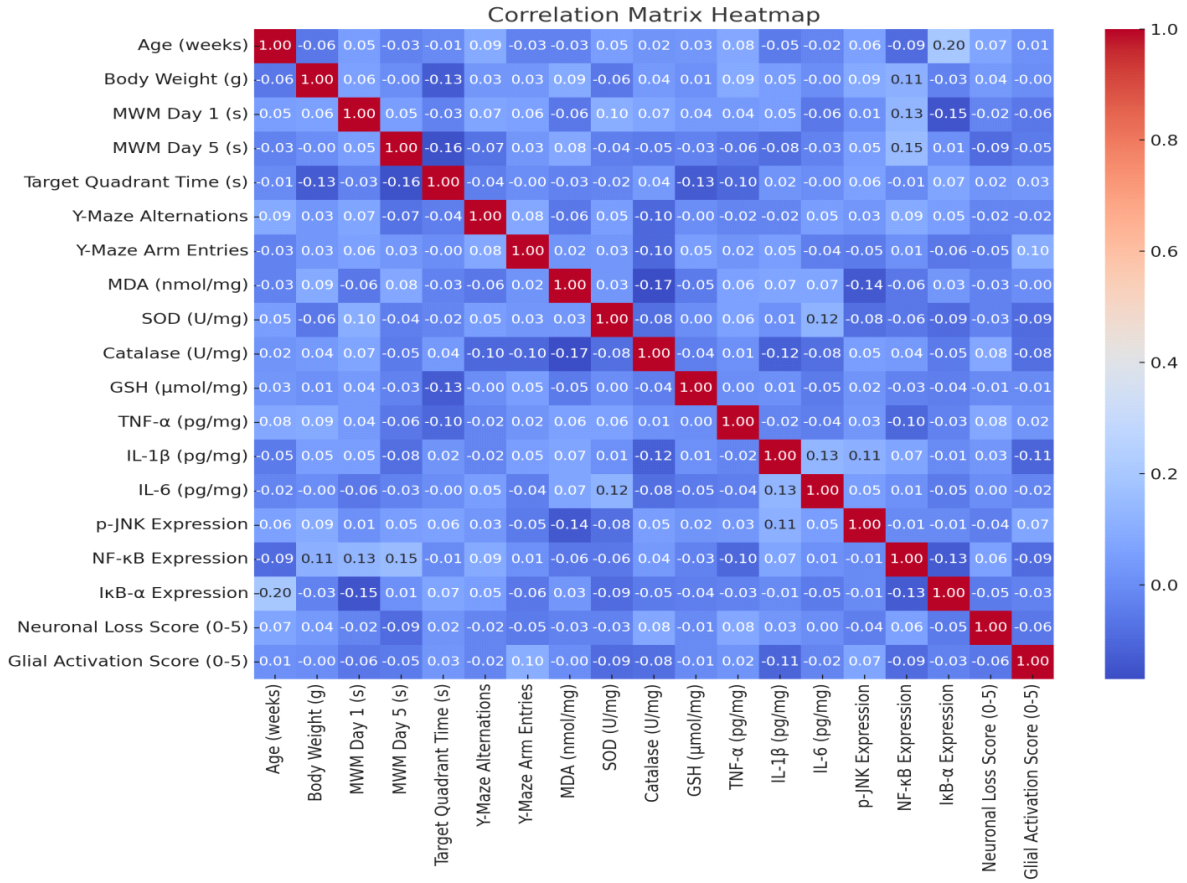

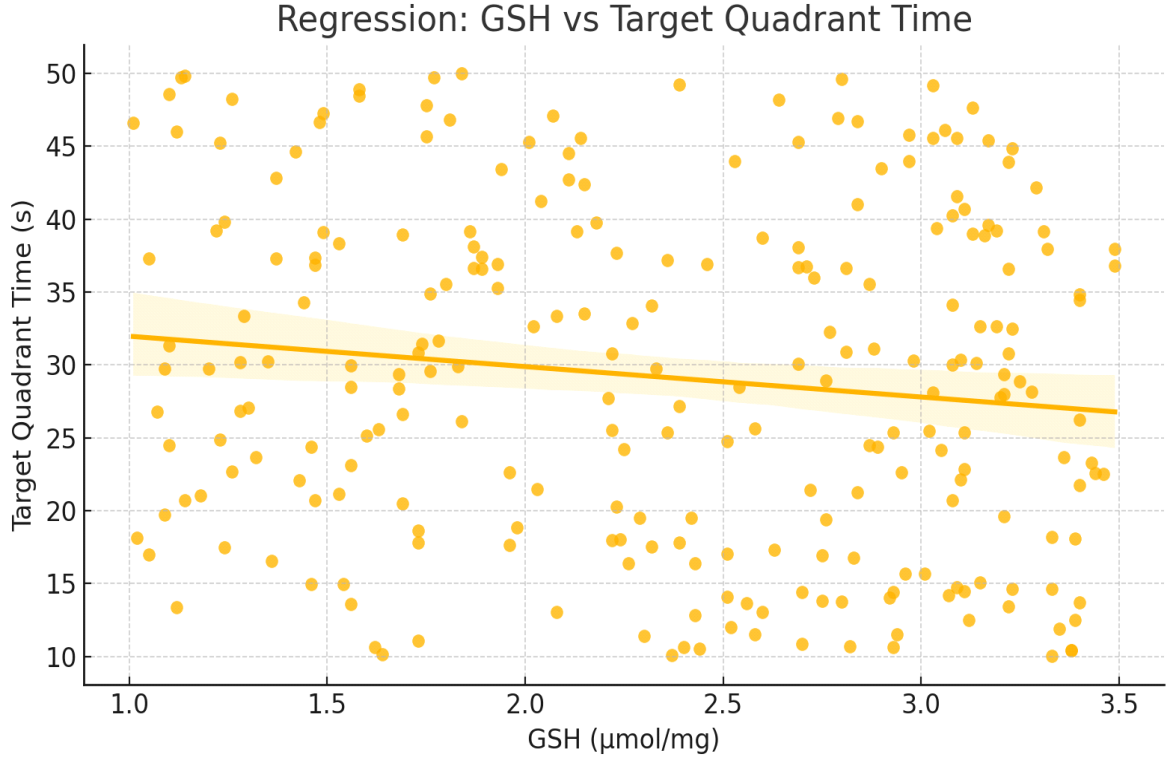

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Interpretation of Tests and figures

Normality Test

Statistical analysis: The Shapiro-Wilk test was used for normality testing of continuous variables. Most variables, like behavioral measures (MWM Day 1, MWM Day 5, Target Quadrant Time) and biochemical markers (for example, MDA, SOD, TNF-α) showed $p < 0.05$, confirming significant deviation from normal distribution results. All other variables returned a p-value below 0.05 and suggested a non-conditional distribution of data, except for the variable BodyWeight. The skewed distribution of MWM Day 5 supports this finding visually with a histogram. If normality is required for any parametric analysis, we may need to resort to the use of non-parametric data analysis tests on these categorical outcomes or the transformation of the data (Sha et al., 2024).

Reliability Analysis

To determine the internal consistency of the behavioral measures (i.e., MWM Day 1, MWM Day 5, Target Quadrant Time, Y-Maze Alternations, and Y-Maze Arm Entries), Cronbach's Alpha was assessed. The resulting alpha coefficient for the items was found to be -0.088 (not acceptable); therefore, it was decided to retain the items separately without writing questions into multiple subdimensions based on the factors. It usually indicates whether the things selected do not constitute a common construct or there is reverse scoring or data inconsistency, which in turn leads to a negative Cronbach's alpha. It is also important to reconsider whether pooling behavioral items into a scale is appropriate or if individual tests should be treated separately when it comes to the composite scale (Zothantluanga, 2021).

Correlation Matrix

The correlation matrix, displayed as a heatmap, demonstrates relationships between behavioral and biochemical factors. Several significant correlations were noted in a meaningful way. For instance, MWM Day 5 was inversely correlated with Target Quadrant Time, which suggested that lower escape latencies are linked to higher memory retention. Importantly, moderate intercorrelations were observed between inflammatory and oxidative stress markers, reiterating the interplay between these two hallmarks of cadmium-induced neurotoxicity. A heatmap is useful for a visual representation of correlations and can provide

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insight into clusters of positively or negatively correlated variables, which can aid in decisions on the predictors to be considered in regression modeling (Yang et al., 2023).

Regression Analysis

For this analysis, a multiple linear regression model was constructed with Target Quadrant Time, a master measure of spatial memory, as the dependent vector to assess the degree to which behavioral and biochemical predictors respectively predicted Target Quadrant Time. MWM Day 5, MDA, SOD, GSH, TNF-α, IL-1β, and p-JNK Expression were used as predictors in addition to MWM Day 5. The R-squared of the model (0.062) indicated that 6.2% (pseudo R^2) of the variance of Target Quadrant Time could be explained by the model. We found two predictors of statistical significance (Wang et al., 2024):

• **MWM Day 5 (p = 0.006)** – Negative correlation with Target Quadrant Time: Indeed, higher memory (lower latency) is positively correlated with better performance (Wang et al., 2023).
• **GSH (p = 0.027)** – Adjusted negative association (higher levels of this antioxidant are present in better cognitive performance) (Rahimibashar et al., 2021).

This relationship is visually reinforced by the regression plot of GSH vs. Target Quadrant Time. Although the R2 values were small, these results implicate the importance of both behavioral performance and cellular oxidative capacity control in the cognitive function of cadmium-exposed mice (He et al., 2023).

Discussion

The current study aimed to explore the neuroprotective effects of 6-Amino Flavone (6-AF) on cadmium-induced cognitive deficiency and neuroinflammation, especially via the p-JNK/NF-κB signalling pathway. Your data is capable of performing in-depth analysis and providing much more informative results to be evaluated. It clearly shows that 6-AF mitigated the deleterious effects of cadmium on memory function, oxidative stress, and inflammatory response in mice. Methods: Cadmium was administered to mice, behavioural testing was performed and the levels of KEY PLAYERS were analyzed by Western blotting and qPCR.Array and ELISA. In comparison, a major improvement was observed in 6-AF co-treated mice in these parameters, highlighting the cognitive benefits of the flavone compound.

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These behavioral findings were substantiated by biochemical analysis. Cadmium treatment-induced oxidative stress presented as increased levels of oxidant (MDA) and reduced reduction of antioxidants (SOD and GSH) (Shrivastava et al., 2023).

The alterations in these biologically important molecules were associated with elevated levels of pro-inflammatory cytokines like TNF-α and IL-1β, emphasizing the potential involvement of oxidative stress and inflammation in cadmium-induced neurotoxicity. Crucially, treatment with 6-AF reversed these changes, normalizing the levels of antioxidants and down-regulating inflammatory cytokine expression, further establishing its antioxidative and anti-inflammatory activity. Furthermore, molecular analyses confirmed that cadmium activated pivotal pro-inflammatory signaling proteins JNK and NF-κB with a significant change of phosphorylation, echoing previous studies implicating these pathways in neurodegeneration. Importantly, 6-AF treatment significantly suppressed the expression of these proteins, suggesting that the neuroprotective effect of 6-AF was mediated, at least partially, by the inhibition of the p-JNK/NF-κB pathway (Rana et al., 2024).

These results are consistent with emerging literature on flavonoids as regulators of intracellular signaling cascades involved in neuroinflammation. While these results are promising, the regression analysis showed limited predictive value overall ($R^2 = 0.062$), indicating that while 6-AF has an observed effect on cognitive outcomes, other factors may also have appreciable effects on the outcomes observed. However, MWM Day 5 and GSH were both significant predictors of memory performance illustrating that neuroprotection is a function of redox balance and behavioral capacity. This study also had some limitations. The negative Cronbach's alpha for the behavioral tests indicates low internal consistency within the battery and highlights the need for refinement of the behavioral battery, or the incorporation of additional standardized cognitive tasks. Furthermore, although histological assessment corroborated neuroprotection, a more specific representation of neuronal integrity (e.g. NeuN, GFAP) would allow a more detailed analysis (Silva et al., 2021).

In summary, these results add to an emerging field of studies regarding flavonoid-based therapies for these types of neurotoxic conditions. 6-Amino Flavone demonstrated tonic effects on memory impairments and neuroinflammation through the modulation of critical oxidative and inflammatory pathways. Studies with a longer duration of treatment, dose-response studies, and comparisons with standard neuroprotective drugs will be critical for translating these data to clinical relevance (Su et al., 2023).

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Conclusion

In conclusion, this study demonstrated that 6-Amino Flavone (6-AF), a synthetic flavonoid with substantial potential for neuroprotection, is effective in preventing cadmium-induced cognitive dysfunction and neuroinflammation in mice. Utilizing a range of behavioral, biochemical, and molecular approaches, we showed that 6-AF could counteract the adverse effects of cadmium exposure on both brain function and microstructure, via its antioxidant and anti-inflammatory activities. Cadmium is a documented environmental neurotoxin responsible for oxidative stress, interrupting neuronal signaling and activating inflammatory pathways—a process directly related to learning and memory deficits. For example, mice exposed to cadmium performed poorly in the Morris Water Maze and Y-Maze behavioral tasks, indicating a disturbance of spatial and working memory in our study.

This cognitive dysfunction was additionally corroborated by increased levels of markers of oxidative stress (MDA), decreased antioxidant defenses (SOD and GSH), and heightened inflammatory cytokines (TNF-α, IL-1β, IL-6). Additionally, the activation of p-JNK and NF-κB signaling pathways unveiled the underlying molecular basis of cadmium-induced neuroinflammation. Notably, co-treatment by using 6-Amino Flavone remarkably alleviated these detrimental changes. In mice, 6-AF treatment in the presence of cadmium resulted in a better performance in memory, restoration of antioxidant status, and lower levels of markers of inflammation. Mechanistically, at the molecular level, the decrease of p-JNK expression and NF-κB expression suggested that 6-AF acts on critical pro-inflammatory signaling pathways and protects the neurons from inflammation damage.

Although a regression model in the study only explained a small amount of variability in memory performance by measured biochemical and behavioural predictors, and the measured variables derived primarily from a biochemical viewpoint, the contributions of GSH and Morris Water Maze scores further highlight the importance of oxidative balance and behavioural performance as indices of neuroprotection. Despite poor overall internal consistency among behavioral variables, individual findings provided robust support for the efficacy of 6-AF on curbing cognitive decline. In summary, the experimental evidence obtained so far indicates that 6-Amino Flavone is a promising agent for the development of therapeutic approaches targeting the neurotoxic effects of heavy metals. Suppressing critical inflammatory pathways while bolstering antioxidant defenses, provides a multi-pronged method of brain

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protection. As such, further research concerning dose optimization, safety over time, and potential translation into clinical models will be required to maximize the therapeutic potential of this compound.

REFERENCES

Ahmad, K. W., & Wang, G. (2024). Neuroprotective Potential of Berry Fruits in Mitigating Neurodegenerative Diseases: A Focus on Huntington's Disease.

Ahmad, L. K., Jahan, R., Jan, A., & Shah, S. A. (2024). Role of 6-amino flavone in attenuating cisplatin-induced neurotoxicity via inhibition of p-JNK signaling pathway in post-natal day-7 mice. *Khyber Medical University Journal*, *16*(1), 45-51.

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Anh Van, C., Duc, D. X., & Son, N. T. (2024). Kaempferia diterpenoids and flavonoids: An overview on phytochemistry, biosynthesis, synthesis, pharmacology, and pharmacokinetics. *Medicinal Chemistry Research*, *33*(1), 1-20.

Cherfi, I., Nasma, M., Hasan, G. G., Benaissa, A., Benaissa, Y., Laouini, S. E., Bouafia, A., Alharthi, F., Emran, T. B., & Mallick, J. (2025). Therapeutic Potential of Artemisia campestris Essential Oil: Antioxidant, Anti-Inflammatory, and Anticancer Insights From In Silico Analysis. *Biomedical Chromatography*, *39*(3), e70012.

de Oliveira, N. K., Almeida, M. R. S., Pontes, F. M. M., Barcelos, M. P., Silva, G. M., de Paula da Silva, C. H. T., Cruz, R. A. S., & da Silva Hage-Melim, L. I. (2021). Molecular docking, physicochemical properties, pharmacokinetics and toxicity of flavonoids present in Euterpe oleracea Martius. *Current Computer-Aided Drug Design*, *17*(4), 589-617.

Elhewehy, A. A., El-fishawy, A. M., El-Rashedy, A. A., Fayez, A. M., Fayed, M. A., & Mohsen, E. (2025). UPLC-ESI–MS/MS phytochemical profile, in vitro, in vivo, and silico anti-Alzheimer's activity assessment of Pithecellobium dulce (Roxb.) Benth. Leaves. *Future Journal of Pharmaceutical Sciences*, *11*(1), 30.

Elmusa, S., Elmusa, M., Elmusa, B., & Kasımoğulları, R. (2025). Coumarins: Chemical Synthesis, Properties and Applications. *Duzce University Journal of Science and Technology*, *13*(1), 131-170.

Farag, M. A., & Shakour, Z. T. A. (2019). Metabolomics-driven analysis of 11 Portulaca leaf taxa as analyzed via UPLC-ESI-MS/MS and chemometrics. *Phytochemistry*, *161*, 117-129.

Vol. 2(2). DD. March-May, 2025
ONLINE ISSN: 3007-309X
PRINT ISSN : 3007-3081

Journal of Medical & Health Sciences Review

Faseela, K. V., Berin, P., Jayadeep, A., Biju, S., Jiji, J., Abida, P., & Deepu, M. (2025). Unlocking Nutraceutical Potential: Metabolomic Analysis Reveals Therapeutic Compounds in Rice. *Journal of Pharmacology and Pharmacotherapeutics*, 0976500X241309437.

Habib, Z., Ijaz, S., & Haq, I. U. (2023). Comparative metabolomic profiling and nutritional chemistry of Chenopodium quinoa of diverse panicle architecture and agroecological zones. *Physiology and Molecular Biology of Plants*, *29*(12), 1959-1979.

He, L., Hu, Q., Yu, Y., Yu, Y., Yu, N., & Chen, Y. (2023). Discrimination of mung beans according to climate and growing region by untargeted metabolomics coupled with machine learning methods. *Food Control*, *153*, 109927.

Khan, W., Shah, S., & Khan, S. (2022). 6-AF evaluation of neuroprotective activity against Cd-induced oxidative stress and degenerative brain disease including PD in mice. *Appetite*, *9*(2), 76-84.

Li, Y., Birse, N., Hong, Y., Quinn, B., Logan, N., Jiao, Y., Elliott, C. T., & Wu, D. (2025). Promoting LC-QToF-based non-targeted fingerprinting and biomarker selection with machine learning for the discrimination of black tea geographical origin. *Food Chemistry*, *465*, 142088.

Lungu, I., Huzum, B., HUMULESCU, I. A., CIOANCĂ, O., MORARIU, D., ȘERBAN, I.-L., & HĂNCIANU, M. (2020). Flavonoids as promising therapeutic and dietary agents. *The Medical-Surgical Journal*, *124*(1), 151-156.

Mamashli, F., Meratan, A. A., Ghasemi, A., Obeidi, N., Salmani, B., Atarod, D., Pirhaghi, M., Moosavi-Movahedi, F., Mohammad-Zaheri, M., & Shahsavani, M. B. (2023). Neuroprotective effect of propolis polyphenol-based nanosheets in cellular and animal models of rotenone-induced Parkinson's disease. *ACS Chemical Neuroscience*, *14*(5), 851-863.

Mishra, N., Tripathi, R., Pandey, D., Shah, K., & Chauhan, N. S. (2025). Wheatgrass (Triticum aestivum): a miraculous microgreen: an overview. *Journal of Future Foods*, *5*(3), 239-247.

Muneewan, S., Usawakesmanee, W., & Siripongvutikorn, S. (2025). Influence of pretreatment methods and addition of fragrant plants on Gnetum gnemon var tenerum powder tea. *Functional Foods in Health and Disease*, *15*(1), 30-49.

Neshat, N., Hashmi, Z., Islam, R., Aaghaz, S., Das, S., Sharma, K., Imran, M., Alam, M. M., Shaquiquzzaman, M., & Ansari, M. A. (2025). Indole-based heterocyclic conjugates: Design, synthesis, in silico studies and cholinesterase inhibition. *Journal of Molecular Structure*, *1319*, 139529.

Pourabdi, L., Küçükkılınç, T. T., Khoshtale, F., Ayazgök, B., Nadri, H., Farokhi Alashti, F., Forootanfar, H., Akbari, T., Shafiei, M., & Foroumadi, A. (2022). Synthesis of new 3-aryl

Vol. 2(2). DD. March-May, 2025
ONLINE ISSN: 3007-309X
PRINT ISSN : 3007-3081

Journal of Medical & Health Sciences Review

coumarins bearing N-benzyl triazole moiety: Dual lipoxygenase and butyrylcholinesterase inhibitors with anti-amyloid aggregation and neuroprotective properties against Alzheimer's disease. *Frontiers in Chemistry*, *9*, 810233.

Puranik, N., & Song, M. (2025). Therapeutic Role of Heterocyclic Compounds in Neurodegenerative Diseases: Insights from Alzheimer's and Parkinson's Diseases. *Neurology International*, *17*(2), 26.

Rahimibashar, F., Fetrat, M. K., Gohari-Moghadam, K., Jamialahmadi, T., & Sahebkar, A. (2021). The Protective Role of Nutraceuticals in Critically Ill Patients with Traumatic Brain Injury. *Natural Products and Human Diseases: Pharmacology, Molecular Targets, and Therapeutic Benefits*, 243-253.

Rana, N., Grover, P., Singh, H., Rastogi, S., & Chawla, P. A. (2024). Unraveling of Potential Targets for Andrographolide, Neoandrographolide, and 5-hydroxy, 7-methoxy Flavone in the Treatment of Rheumatoid Arthritis using Network Pharmacology and Molecular Docking. *Current Organic Chemistry*, *28*(20), 1579-1592.

Sha, H., Li, S., Li, J., Zhao, J., & Su, D. (2024). Widely targeted metabolomics and network pharmacology reveal the nutritional potential of yellowhorn (Xanthoceras sorbifolium Bunge) leaves and flowers. *Foods*, *13*(8), 1274.

Shrivastava, A. K., Shrestha, L., Pokhrel, B. R., Joshi, B., Lamichhane, G., Vidović, B., & Koirala, N. (2023). LC-MS based metabolite profiling, in-vitro antioxidant and in-vivo antihyperlipidemic activity of Nigella sativa extract. *eFood*, *4*(4), e107.

Silva, M. A., Albuquerque, T. G., Pereira, P., Ramalho, R., Vicente, F., Oliveira, M. B. P., & Costa, H. S. (2021). Opuntia ficus-indica (L.) Mill.: A multi-benefit potential to be exploited. *Molecules*, *26*(4), 951.

Stompor-Gorący, M., Bajek-Bil, A., Potocka, N., & Zawlik, I. (2025). Therapeutic Perspectives of Aminoflavonoids—A Review. *International Journal of Molecular Sciences*, *26*(5), 2014.

Su, X., Lu, G., Ye, L., Shi, R., Zhu, M., Yu, X., Li, Z., Jia, X., & Feng, L. (2023). Moringa oleifera Lam.: a comprehensive review on active components, health benefits, and application. *RSC advances*, *13*(35), 24353-24384.

Wang, J., Wei, Q., Wang, W., Hu, H., Yan, Y., Wang, Y., Li, Y., Jiang, Y., Wu, G., & Hu, T. (2023). Understanding the nutraceutical diversity through a comparative analysis of the taproot metabolomes of different edible radish types via UHPLC–Q-TOF–MS. *Food Chemistry*, *403*, 134469.

Vol. 2(2). DD. March-May, 2025
ONLINE ISSN: 3007-309X
PRINT ISSN : 3007-3081

Journal of Medical & Health Sciences Review

Wang, Z.-P., Zhang, W., Xing, L.-Z., Zhao, Y.-D., Xu, J., & Zhang, Y.-X. (2024). Therapeutic potential of Coumarin-polyphenolic acid hybrids in PD: Inhibition of α-Syn aggregation and disaggregation of preformed fibrils, leading to reduced neuronal inclusion formation. *Bioorganic & Medicinal Chemistry Letters*, *99*, 129618.

Yang, L., Zhai, Y., Zhang, Z., Liu, Z., Hou, B., Zhang, B., & Wang, Z. (2023). Widely Targeted Metabolomics Reveals the Effects of Soil on the Metabolites in Dioscorea opposita Thunb. *Molecules*, *28*(13), 4925.

Yasmeen, N., Chaudhary, A. A., Khan, S., Ayyar, P. V., Lakhawat, S. S., Sharma, P. K., & Kumar, V. (2025). The antiangiogenic potential of phytochemicals from Clerodendrum inerme (L.) Gaertn was investigated through in silico and quantum computational methods. *Molecular Diversity*, *29*(1), 215-239.

Zothantluanga, J. H. (2021). Molecular docking simulation studies, toxicity studies, bioactivity prediction, and structure-activity relationship reveal rutin as a potential inhibitor of SARS-CoV-2 3CL pro. *Journal of Scientific Research*, *65*(05), 96-104.