OPEN ACCESS

ORIGINAL ARTICLE *Volume 14 (Issue 4), 2025*

PAKISTAN JOURNAL OF MEDICINE & DENTISTRY

CROSSLINKING APIGENIN WITH NEURODEVELOPMENT: IN–VIVO MODEL DESIGNING BASED THERAPEUTIC STRATEGY FOR AUTISM–ASSOCIATED NEUROINFLAMMATION

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ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder characterized by impaired social interaction, communication difficulties, and repetitive behaviors. Neuroinflammation is increasingly recognized as a key contributor to ASD pathophysiology. Nerve Growth Factor (*NGF*), a vital neurotrophin involved in neuronal survival and plasticity, is often dysregulated in ASD, exacerbating neurological dysfunction. Apigenin, a natural flavonoid with known anti-inflammatory and neuroprotective properties, has shown promise as a therapeutic agent in neurodevelopmental conditions. This study evaluated the effects of apigenin treatment on autism-like neuroinflammation by examining its impact on *NGF* regulation in a rodent model.

Methods: This in vivo study (July–November 2021) used 20 healthy male rats (8 weeks old). The research took place within the Animal House facility and was analyzed at SMDC Lahore and LUMHS Jamshoro, Sindh. Group I (n=4) was taken as control, while Groups II–V (n=16) received 250 mg/kg/day propionic acid (PPA) to induce autism-like neuroinflammation. Groups III–V were treated with Apigenin at 50, 100, and 200 mg/kg. *NGF* serum levels were analyzed using the enzyme-linked immunosorbent assay (ELISA). SPSS v 21 was used for statistical analysis using one-way ANOVA and Tukey's test. $p<0.05$ was taken as significant.

Results: The levels of *NGF* were significantly lowered using propanoic acid (PPA) (4.3 ± 0.5 pg/mL vs control 11.9 ± 0.5). Apigenin dose-dependently restored *NGF*, with Group V (200 mg/kg) reaching 9.8 ± 0.5 pg/mL.

Conclusion: The neuroprotective properties of apigenin are evident through its ability to restore PPA-damaged levels of *NGF*, thus establishing potential use as an autism treatment for neuroinflammation in neurodevelopmental disorders.

Keywords: Apigenin, Autism, Neuroinflammation, *NGF*, Neurodevelopment, In-Vivo Model.

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Citation: Shaikh P, Memon SG, Tanvir S, Iqbal W, Mehmood A, Nangdev P Crosslinking Apigenin with Neurodevelopment: In-Vivo Model Designing based Therapeutic Strategy for Autism-Associated Neuroinflammation. Pak J Med Dent. 2025 September ;14(4): A-B. Doi: https://doi.org/10.36283/ziun-pjmd14-4/002.

Received: Mon, April 21, 2025 **Accepted:** Fri, August 15, 2025 **Published:** Mon, September 29, 2025

INTRODUCTION

Autism Spectrum Disorder (ASD) is a developmental disorder that results in lasting social impairment, communication deficits, and behavioral abnormalities, which first appear during early childhood [1]. Recent demographic statistics showed that ASD affects 1 in 54 American children, with boys experiencing ASD more commonly than girls [2]. The exact causes of ASD remain unidentified, yet experts agree that genetic risk, environmental exposures, and neuroinflammation are contributing factors in its development [3]. Recent studies showed that brain inflammation has become a significant risk factor for ASD development, specifically when it occurs in vital stages of brain development [4]. Research has greatly intensified to determine the presence of bioactive compounds that work both as anti-inflammatory substances while providing neuroprotective benefits [5].

Researchers focused on Apigenin, which occurs naturally as a flavonoid compound in chamomile, parsley, celery, and other plant varieties. It presents strong neurotrophic, antioxidant, and anti-inflammatory properties when tested through in vitro and in vivo model systems [6]. Researchers found Apigenin particularly worth investigating due to its influence on the vital neurotrophin Nerve Growth Factor (*NGF*), which helps develop, maintain, and protect brain cells [7]. Neurodevelopment and synaptic plasticity are controlled by *NGF*, which gets disrupted during ASD cases [8].

Studies on autism models showed that inadequate *NGF* regulation leads to problems with neuronal communication and cognitive function. Restoration of *NGF* levels may offer a potential remedy for autism symptoms [9,10]. Therefore, a study was required for this purpose.

This study evaluated how apigenin treatment affects autism-related neuroinflammation through assessment of its impact on Nerve Growth Factor (*NGF*) regulation. To assess whether Apigenin functions as a treatment for autism-associated neuroinflammation, researchers are developing an in vivo care model for this research.

METHODS

The in vivo study (EC/0785/22/) was conducted from July 2021 to November 2021. The animal-based experiments were compiled with the protocols for institutional animal care and use. The research took place within the Animal House facility and was analyzed at SMDC Lahore and LUMHS Jamshoro, Sindh. This study relied on twenty male rats that were eight weeks old, with weights between 180–220 grams. All rats were healthy. The animals were housed under Standard Temperature and Pressure (STP) environmental conditions during experiments through which research took place with a constant room temperature of 22 ± 2°C, humidity ranging from 40–60%,

and a 12-hour light/dark cycle regulating daily cycles. The animals received unlimited standard laboratory chow and water throughout the experimental period. General sanitation practices involved routine cleaning procedures and sterilization to maintain ideal health standards across all cages.

The experimental animals were randomly distributed into five distinct groups, with four subjects assigned to each group. The physiological control group (Group I) received neither PPA administration nor experimental treatment. Group II: Autism-induced model via Propionic Acid (PPA). PPA-treated animals received oral doses of Apigenin at 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight under Groups III–V. Autistic-like neuroinflammatory conditions were chemically induced in Groups II–V through daily intraperitoneal injections of Propionic Acid (PPA) at 250 mg/kg/day for five days continuously, while normal saline served as the dilution medium. The administration of Apigenin at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg occurred once daily for a total span of four weeks following autism induction. The laboratory team prepared Apigenin fresh using a proper vehicle to achieve precise administration volumes.

The study collected blood samples before processing the serum to analyze *NGF* levels. A standardized enzyme-linked immunosorbent assay protocol operating on an automated ELISA reader identified *NGF* levels through a series of manufacturer-provided operation procedures from reagent preparation through sample loading and optical density measurements. The SPSS Version 21 software processed the obtained data. The results appeared in the form of mean values with standard deviation (SD). Statistical significance between groups was assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The ELISA assay revealed significant variations in *NGF* levels among the experimental groups ($p < 0.05$). Propionic acid administration markedly reduced *NGF* concentrations in Group II compared to the control. To confirm the development of the in vivo model, this study assessed the *NGF* levels across five experimental groups using the ELISA method. Table 1 demonstrates the conditions of animal husbandry in which rodents were nourished for this study. It included species, age, weight, conditions, sample size, study period, ethical approval, and facilities in which this study was performed.

Table 1: Animal Husbandry and Husbandry Conditions

Parameter	Value
Species / Strain	Wistar rats (male)
Age at start	8 weeks
Baseline weight range	180–220 g
Temperature	22 ± 2 °C
Humidity	40–60 %
Light / Dark cycle	12 h / 12 h
Food & Water	Ad libitum
Group size	4 rats per group
Study period	July–November 2021
Ethical approval	EC/0785/22
Facilities	Animal House at SMDC & LUMHS

Table 2: Quantitative Measurements of Luteolin's Therapeutic Potential, Increased *NGF* (pg/ml) levels

Groups (Parameter)	I (Control)	II (PC)	III (APG-50)	IV (APG-100)	V (APG-200)	Statistics (*P-value*)
BDNF Levels (ng/ml)	13.1 ±0.3	5.1 ± 0.2	9.8 ± 0.3	8.0 ± 0.3	10.1 ± 0.3	0.05
Doses and Treatments	On a regular diet only	On PPA only	100mg/kg Luteolin treatment	200mg/kg Luteolin treatment	400mg/kg Luteolin treatment	-

The table shows cumulative mean values + standard error of *NGF* levels (pg/ml). The NGF levels are paralleled with the positive control group, with maximum recovery in NGF levels of group V in ng/ml (10.1±0.3), as shown in **Table 2. Groups: I (Control):** Non-induced control group, **II (PPA):** Propionic acid-induced group, **III (APG-50):** Apigenin 50 mg/kg, **IV (APG-100):** Apigenin 100 mg/kg, **V (APG-200):** Apigenin 200 mg/kg.

Figure 1 presents the mean *NGF* concentrations, calculated by integrating serum results from each investigated group. Neuroinflammatory damage in autism-like pathology resulted from the lower *NGF* concentrations induced by propionic acid (Group II) compared to the non-induced control group (Group I). *NGF* levels decreased in a dose-dependent manner with Apigenin treatment, starting from Group III through V, and reached near-baseline values in Group V at a dosage of 200 mg/kg. Apigenin treatment effectively restored PPA-induced deficits in *NGF* levels, as the groups displayed statistically relevant variations ($p < 0.05$) between each other. The *NGF* levels of Group V were at

levels comparable to the control group, which supports the Neuroprotective properties of Apigenin through its action on *NGF* regulation.

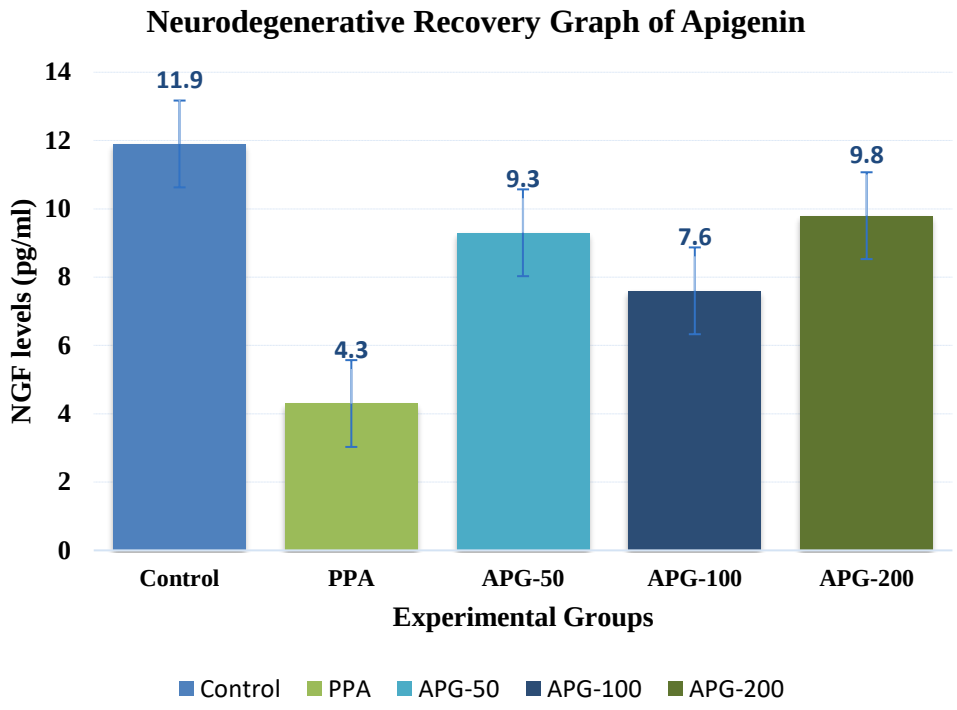

Figure 1: Comparative Analysis of Serum *NGF* Levels Across Experimental Groups

The examined data, through graphs, show that Apigenin treatment induces elevated levels of *NGF*, which confirms its ability to counter autism-mediated inflammation of neural tissue.

DISCUSSION

The pathophysiological examination of autism spectrum disorder (ASD) requires new therapeutic approaches, as current research emphasizes neuroinflammation and dysregulated neurotrophin signaling as key biological pathways. PPA-induced models establish themselves as a standard approach for simulating autistic features by inducing neuroinflammation, thereby enabling valid testing of potential neuroprotective agents. This study investigated the therapeutic properties of Apigenin, a bioactive flavonoid, to control neuroinflammation through its management of serum *NGF* levels, which are crucial for neural development and synaptic function. The study data proved that PPA treatment produced substantial *NGF* reduction patterns in rat subjects, which aligned with ASD-related developmental problems [11,12]. The highest tested dose of Apigenin at 200 mg/kg led to *NGF* value normalization, comparable to the levels seen in control conditions.

Scientific evidence demonstrated that Apigenin acts as a neuroprotectant because it reduces inflammation and acts as an antioxidant while modifying neurotrophic signaling pathways [13,14]. Research using Apigenin on living systems and petri dish models demonstrated its ability to modify inflammatory cascade mediators and promote neuronal health, which may explain why this finding emerged in our experimental model [15]. Extensive research indicated that Apigenin administration improves *NGF* levels during neurotoxic PPA treatment, making this flavonoid a significant strategy for fighting neuroinflammatory damage and triggering neuroregenerative processes [16,17]. Predicted results showed that *NGF* supports the proper functioning of nervous system cells while maintaining their structural integrity. It is also directly associated with ASD manifestations, including autism spectrum disorders disabling characteristics. As a natural *NGF*-targeting agent, Apigenin demonstrates potential for treating core autism features [18,19].

The statistical analysis showed substantial variations ($p < 0.05$) between *NGF* levels among the groups, thus validating the therapeutic benefits of Apigenin. This study strengthens the value of plant extracts as remedies for neurodevelopmental disorders by promising their implementation in ASD research development, as demonstrated in previous studies [20,21]. Future studies must evaluate several critical aspects that demonstrate the promising therapeutic potential of Apigenin in reversing PPA-induced neuroinflammation and regulating *NGF* levels. The necessary step should include observing behaviors that correspond with modified *NGF* concentrations to show how these biochemical changes affect autism-related conduct [22,23]. Social interaction assessments and repetitive behavior evaluations should be integrated to determine how the research results relate to actual clinical application.

The antioxidant properties of Apigenin deserve additional research on oxidative stress markers because they could supply further defense against neuroinflammatory damage. The evaluation of MDA, GSH and SOD activity as biochemical markers would help establish if Apigenin diminishes oxidative stress in addition to reducing inflammation [24,25]. Long-term studies are necessary for evaluating both the safety and the most effective dosage and chronic efficacy of Apigenin because this research depended on a 4-week testing period only. Long-term research needs to investigate Apigenin's role as an intervention tool for ASD treatment since ASD requires ongoing therapeutic approaches.

CONCLUSION

Apigenin showed effectiveness in restoring *NGF* levels to normal in rodent models of autism-related neuroinflammation, according to this study, thus reinforcing its capability to protect neural cells in ASD-related biochemical imbalances. The research data showed that Apigenin presents value as a medicinal plant compound that exhibits both anti-inflammatory and neurotrophic activity in treating autism spectrum disorders and neurological defects. The findings support existing scientific evidence that promotes natural approaches to regulate neurotrophins for treating ASD-related neuropathology.

LIST OF ABBREVIATIONS

ASD: Autism Spectrum Disorder
ANOVA: Analysis of Variance
APG: Apigenin
ELISA: Enzyme-Linked Immunosorbent Assay
IP: Intraperitoneal
***NGF*:** Nerve Growth Factor
PPA: Propionic Acid
STP: Standard Temperature and Pressure

ACKNOWLEDGMENT

None

FUNDING

None

CONFLICT OF INTEREST

None

ETHICAL APPROVAL

The study certificate SOPs were permitted by Suleman Roshan Medical College and LUMHS Jamshoro collaboratively (EC/0785/22).

AUTHORS' CONTRIBUTION

All authors have equal contributions as per ICMJE.

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