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" *Quercetin alleviates Valproic Acid-Induced Autistic Features in mice via down-regulation of TLR4/NF-κB signaling pathway* "

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ABSTRACT:

Introduction: Autism spectrum disorder (ASD) is linked to stereotypical behavior and poor social skills. Quercetin (Qrct) has been shown to possess anti-inflammatory and antioxidant impacts.

Objective: to demonstrate the underlying processes and the neuroprotective impact of Qrct in VPA-induced ASD.

Material & methods: Thirty male Wister albino rats were split into three: control, VPA, and VPA+ Qrct. Following neurobehavioral testing, the rats were sacrificed, and the cerebellar gene expression of TLR4 and NF-kB was evaluated together with the measurements of cerebellar MDA, SOD, TNF-α, IL-6, IL-10, BDNF, and serotonin. Cerebellar and histopathological immunoreactions for Bax and NF-kB were performed.

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Results: While the VPA group's number of crossing slots in OFT, time of central crossing in OFT, cerebellar SOD, cerebellar IL-10, cerebellar BDNF, and cerebellar serotonin were dramatically decreased than those of the control, the VPA group's rearing frequency in OFT, time in the open arms of EPM, cerebellar MDA, cerebellar TNF-α, cerebellar IL-6 and cerebellar TLR4 and NF-kB gene expression dramatically increased than those of the control. Additionally, the VPA group's NF-kB and Bax cerebellar immunoreaction were dramatically increased than those of the control. Qrct significantly enhanced ASD caused by VPA.

Conclusion: Qrct reduced VPA-induced ASD by down-regulation of the cerebellar TLR4/NF-kB pathway as well as anti-oxidant, anti-inflammatory, neurotropic, and antiapoptotic pathways.

Key words: Autism, Bax, BDNF, NF-kB, Quercetin, TLR4

Introduction

A complex neurological condition, autism spectrum disorder (ASD) presents as altered social interaction, restricted interests, and behaviors. ASD typically manifests throughout the first years of life. An estimated 1 in 54 people have ASD. The prevalence

is four times higher in males. ASD is a very upsetting illness that has reached pandemic proportions despite all efforts to understand it. Its pathogenic underpinnings are still unknown. In the pathophysiology of ASD, altered immune function and modulation have been highlighted (Farrag et al., 2024).

Even while genetics shares in the development of ASD, it has been demonstrated that a variety of genetic, environmental, immunological, and neurological variables interact to cause the disorder. ASD has also been connected to oxidative stress, mitochondrial malfunction, immunological disorders and inflammation (Ayaydin et al., 2020).

Drugs or environmental irritants at critical developmental phases results in ASD. ROS are produced by chemicals, are the source of inadequacies in the limbic system, cerebellum, and brain development. (Pham et al., 2022).

ASD is characterized by biochemical abnormalities, including aberrant redox state, mitochondrial dysfunction, and the activation of the inflammatory cascade. According to recent research, autism causes major disruptions in brain activities by affecting certain brain regions. The pathophysiology of autism may be significantly influenced by cerebellar impairment. Defects in motor behavior and cognition are linked to these anomalies (Alnakhli et al., 2024).

It is commonly known that the antiepileptic drug valproic acid (VPA) causes fetal valproate syndrome, which presents as hyperexcitability, language and communication impairments, behavioral delays, and stereotypical behaviors. (Markram et al.2008).

Giving VPA to mice within the first 14 days of life disrupts neurodevelopmental processes, leading to autism-like behavioral impairments. PND (postnatal day) 14 is a crucial period for neuronal differentiation, synaptogenesis, and gliogenesis in the cerebellum. It causes neurodevelopmental regressions that affect behavior (Molinari et al., 2025).

In terms of development and survival, BDNF is essential. It supports neuronal survival and differentiation, synaptic plasticity, and all of these aspects uphold neuroprotection. In order to address the synaptic abnormalities and cognitive dysfunctions linked to ASD, BDNF helps to strengthen the characteristics of synaptic connections (Khan et al., 2024).

Both innate and adaptive immunity are heavily regulated by toll-like receptor (TLR) signaling, which also contributes to the neuroinflammatory development in models of ASD. Due to its activation of ROS and inflammatory cytokines, TLR-4 is the primary

cause of ASD. TLR-4 signaling triggers NF-κB pathway. TLR4 and NF-κB levels are higher in autistic children, and ROS signaling is also elevated. Excess ROS causes apoptosis by destroying proteins, lipids, membranes, organelles, and nucleic acids at the cellular level. ROS signals trigger apoptosis and increase the synthesis of inflammatory genes. The development of new neuroprotective medications that modulate autism is crucial to the ongoing enhancement of ASD characteristics (Farrag et al., 2024). It has also been noted that in animal models of neurodegenerative illnesses, a mutation in TLR4 causes motor impairments (Molinari et al., 2025).

ASD is linked to changes in serotonergic systems (Rossignol, & Frye, 2012). Chronic inflammation is important in neurological conditions. Its function is not restricted to certain neurotransmitter disorders; it also results in modifications to the brain's oxidative/antioxidant status, immunological response, endocrine system, and neurotransmitter circuits (James et al., 2006).

ASD does not currently have a cure. The only symptoms of ASD that can be managed with current medications are anxiety, hyperactivity, seizures, obsessive behavior, and digestive issues (Yang et al., 2020).

One type of plant flavonoid, quercetin (Qrct), is found in a variety of plants and plant-based foods, including berries, red wine and onions. By controlling enzymes and transcription factors in the inflammatory signaling cascade, it has anti-inflammatory properties in addition to antioxidant and free radical scavenger actions. Moreover, cerebellar inflammation was reduced by Qrct therapy. By blocking the TLR4/NF-κB signaling pathway, Qrct may have had a neuroprotective effect on brain damage (Wu et al., 2019).

Because it can alter the majority of signaling pathways, lower oxidative stress levels, and prevent neuroinflammation, Qrct has a potent neuroprotective profile. BDNF levels are also impacted by Qrct (Khan et al., 2024)..

The aim of this study was to investigate the neuroprotective effects of Qrct on ASD caused by VPA and potential underlying mechanisms including referral to the TLR4/NF-kB signaling pathway.

Materials and methods

Animals

Theodore the Bilharz Research Institute (Giza, Egypt) provided thirty male Swiss mice weighing 15–25 g, which were acclimated. They were kept in environments with controlled temperature, They had unrestricted access to water and regular mouse food. The Institutional Animal Care and Use Committee (IACUC), Menoufia University, with IRB No: MUFS/F/GE/4/25 approved the research

Experimental design

Following the acclimatization period, mice were divided into three groups of ten at random.

(1) Control group: Starting with PND14, mice were given 1 mL of distilled water intragastrically once day for four weeks.

(2) Valproic acid-treated group (VPA): On PND 14, mice received a subcutaneous injection of 400 mg/kg sodium valproic acid (Sigma Aldrich, St. Louis, MO, USA). They also received 1 mL of distilled water intragastrically once daily for four weeks (Pragnya et al., 2014).

(3) Valproic acid + Quercetin group (VPA + Qrct): On PND14 mice were given VPA at a dose of 80 mg/kg/day, diluted in 1 mL of distilled water, and Qrct (Sigma-Aldrich Co., Mo, USA), orally gavaged once daily for four weeks starting from the 1st day of the study. (Kılıç et al., 2025).

24 hours following the conclusion of the intervention, the behavior of every mouse was assessed. Lastly, xylazine and ketamine anesthesia (10 and 35 mg/kg, respectively) were used to induce the euthanasia procedure. This was followed by brain dissection, cervical dislocation, and decapitation. The right half of the cerebellum was preserved for histological and immunohistochemical investigation using 10% buffered formalin, while the left half was split evenly for biochemical study and RT-PCR assays.

Neurobehavioural Tests

Open field test: Mice were housed in a Plexiglas arena (30 x 20 cm) with 12 equal-sized squares for five minutes. Recorded were the number of crossing slots, rearing movements, and center crossing times (Silverman et al., 2010).

Elevated Plus Maze (EPM) Test: As (Tchantchou et al., 2018), In this experiment, we used an apparatus to have the rats identify a plus sign in order to assess their anxiety-like behavior. Each rat was placed in the middle of the apparatus and allowed ten minutes to explore the labyrinth. The animals' movements were monitored by an overhead security camera. The duration of time spent in the open arms mazes was meticulously documented. The duration and the intensity of anxiety-like behavior were inversely correlated.

Social Approach (Three-Chamber) Test: The test was administered in two 10-minute sessions. A mouse was positioned in the middle of a Plexiglass box that was separated into three interconnected compartments during the first session. It was given the option to engage with an empty wire cup in one side chamber or a similar wire cup with an unknown mouse (stranger I) in the opposite chamber after five minutes of habituation. The mice were matched in terms of strain, age, and sex. The amount of time spent using each cup was tracked. In the second session, put a second control mouse (Stranger II) in the other side chamber of the same wire confinement cup. It should be identical in terms of strain, age, and sex. The amount of time spent using each cup was recorded (Peñagarikano et al., 2015).

Tissue Homogenate Preparation

Each weighted cerebellar tissue was homogenized separately. The homogenate was centrifuged for 11 minutes at 15,000 rpm. The supernatant was collected and stored. Cerebellar TNF-α (Cat.: MBS2507393, MyBioSource, Sandiego, CA, USA), cerebellar IL-6 (Cat.: MBS269892, MyBioSource, Sandiego, CA, USA), cerebellar IL-10 (IL-10: ERI3010-1, Assaypro LLC, Saint Charles, Missouri, USA), cerebellar BDNF (Cat. No. MBS355435, MyBiosource, Sandiego, CA, USA), and cerebellar serotonin (Cat. No. MBS160104, MyBiosource, Sandiego, CA, USA) were measured using the ELISA Kit in accordance with the manufacturer's instructions. Calorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt) were used to assess cerebellar MDA and superoxide dismutase (SOD).

Quantitative RT-PCR

One piece of cerebellar tissue was taken from each rat and placed in a falcon tube, where it was kept at -80 °C for RNA extraction and the TLR4 and NF-kB assay. A 7500 real-time PCR machine (Applied Biosystems, CA, United States) was used to identify TLR4 and NF-kB. The first step of PCR was the synthesis of complementary DNA using the QuantiTect Reverse Transcription Kit (205311; Qiagen, Applied Biosystems, USA), which was followed by the second step of PCR after RNA was extracted from cerebellar cells using a direct—zol RNA miniprep kit (Cat. No. R2051; Zymo Research, USA). The following primers were used

The NF-kB forward primer was (TCGACCTCCACCGGATCTTTC). The reverse primer was (GAGCAGTCATGTCCTTGGGT). The forward primer for TLR4 was (TCAGCTTTGGTCAGTTGGCT), and the reverse was (GTCCTTGACCCACTGCAAGA). Actin works as an endogenous control. Ten microliters of SYBR Green (2× QuantiTect PCR Master Mix), three microliters of cDNA, one microliter of forward primer, one microliter of reverse primer, and five milliliters of RNase-free water were used in each PCR reaction, which was carried out in a final volume of 20 microliters. Denaturation at 94 °C for 30 s, annealing at 55 °C for 40 s, and extension at 72 °C for 31 s were the next 55 cycles. The data was processed using the Applied Biosystems 7500 software version 2.0.1. Gene expression was measured relative to one another using the comparative Ct technique. The TLR4 and NF-kB genes' melting curve and amplification plot (Khodir et al., 2025).

Histopathological method

The cerebellum was embedded in paraffin wax after being preserved in formalin. Deparaffinized 5-µm slices were subjected to histopathological analysis. These were then successively rehydrated using ethanol grades of 100%, 90%, and 70%. The dye Hematoxylin and Eosin was applied.

For immunohistological staining 5-µm slices were blocked in 0.1 H_2O_2 % ,, for 30 minutes after being washed with PBS .The slices were rinsed in PBS and then incubated at room temperature for 60 minutes in blocking solution) After that, the sections were treated for an hour at room temperature with the primary antibodies anti-Bax (rabbit polyclonal ,Sigma-Aldrich) and anti-NF-kB) monoclonal ,dilution 1:200 ,Abcam)

For quantitative assessment Five separate sections (400 X) were analyzed using Image J software (1.74v) in order to calculate the number of PCs and the area % of Bax and NF-kB.

Statistical analysis

Following data collection and analysis, they were found to satisfy the parametric assumptions based on the results of the Shapiro-Wilk test. As a result, one-way ANOVA and post hoc Bonferroni's tests were applied to the data. The data was displayed using the mean ± SD. Significance was considered to exist when the p value was 0.05 or less. The data was analyzed using Graph-Pad Prism software (version 9.3.1, San Diego, CA, USA).

Results

While the VPA group's number of crossing slots in OFT, time of central crossing in OFT, cerebellar SOD, cerebellar IL-10, cerebellar BDNF, and cerebellar serotonin were dramatically lower than those of the control, the VPA group's rearing frequency in OFT, time in the open arms of EPM, cerebellar MDA, cerebellar TNF-α, cerebellar IL-6, cerebellar gene expression of TLR4, and cerebellar gene expression of NF-kB were all dramatically higher than control. The VPA + Qrct group had significantly higher levels of the number of crossing slots in OFT, the time of central crossing in OFT, the cerebellar SOD, the cerebellar IL-10, the cerebellar BDNF, and the cerebellar serotonin than the VPA, but dramatically lower levels of the rearing frequency in OFT, time in the open arms of EPM, cerebellar MDA, cerebellar TNF-α, cerebellar IL-6, cerebellar gene expression of TLR4, and cerebellar gene expression of NF-kB.. Table (1).

Table (1): The measured OFT results, Time in the open arms of EPM, Cerebellar MDA, Cerebellar SOD, Cerebellar TNF-α, Cerebellar IL-6, Cerebellar IL-10, cerebellar BDNF, Cerebellar Serotonin, Cerebellar TLR4 gene expression and Cerebellar NF-kB gene expression in all studied groups

	Control group	**VPA group**	**VPA +Qrct group**
Number of crossing slots in OFT	150.8±4.3	50.8±6.3 *	**107.9±4.2***#
Time of central crossing in OFT	30.8±1.9	14.5±2.1 *	**23.8±1.7** *#
Rearing frequency in OFT	20.5±2.1	63.5±4.2 *	**47.9±2.6** *#
Time in the open arms of EPM	120±4.6	72±5.3 *	**98.6±6.1** *#
Cerebellar MDA (nmol/ gm. Tissue)	7.8±1.54	31.8± 3.87*	**17.9± 2.55***#
Cerebellar SOD (U/gm. Tissue)	9.8±1.01	2.33±0.6*	**6.33±0.9***#
Cerebellar TNF-α (pg/ml)	70.8±3.9	250.9±6.35*	**144.9±3.8***#
Cerebellar IL-6 (pg/mL)	153.9±7.8	410±6.58*	**274±6.3***#
Cerebellar IL-10 (ng/mL)	11.8±0.11	4.38±0.31*	**6.99±0.4***#
Cerebellar BDNF (pg/ml)	258.3±11.4	97±6.88*	**193.9±7.13***#
Cerebellar Serotonin (ng/ml)	163.8±3.8	71.9±2.58*	**114.9±3.15***#
Cerebellar TLR4 gene expression	1	3.25±0.07*	**2.77±0.05***#
Cerebellar NF-kB gene expression	1	2.33±0.03*	**1.85±0.08***#

* Significant compared with control, # Significant compared with VPA.

In the first session, it was shown that the time spent by the subject mice in the control group with stranger was considerably greater ($p<0.05$) than the time spent by the subject mice in the empty chamber. Mice in the VPA, however, spent almost the same amount of time in the stranger and empty chambers, indicating no preference for social proximity. The amount of time spent with the stranger mouse was dramatically higher ($p<0.05$) in the VPA+Qrct than the amount of time spent in the empty chamber. The mice in the control group spent much more time with stranger II than with stranger I during the second session. However, mice in the VPA showed no preference for social

proximity, spending nearly the same amount of time in the strangers I and II chambers. In the VPA+Qrct group, the duration of time spent with stranger II was noticeably longer than that of the stranger I chamber. (Table 2)

Table (2): Three chamber test results in all studied groups

Groups	Control		VPA		VPA+Qrct	
	empty	Stranger	empty	Stranger	empty	Stranger
Session I	136.8±4	403±6	141±7	144±6.8	137±8.3	305.8±6.4
	Stranger I	Stranger II	Stranger I	Stranger II	Stranger I	Stranger II
Session II	70.9±3.8	420.8±7.8	155±4.6	165.9±6.8	110±8.9	330±6.98

Histological results:

H&E

Three layers made up the control group's cerebellum: the inner granular, the middle Purkinje cell layer (PC) was arranged in a single, and the outer molecular layer was primarily composed of fibers with a few glial cells.

The VPA group seemed smaller and displayed obvious neuronal affection, particularly at the PC layer, which showed noticeable disarray. In comparison to the control, there were considerably fewer PCs (5.93±0.14 vs. 16.25±0.46, $p<0.05$). The granular layer was also made up of closely spaced cells. Dispersed, strongly pigmented pyknotic cells were visible in the molecular layer. Comparing the VPA-Qrct group to VPA, there was a significant upregulation of the PCs and an evident improvement in the three layers of the cerebellum (11.33±1.34 vs. 5.93±0.14, $p<0.05$). (Fig 1)

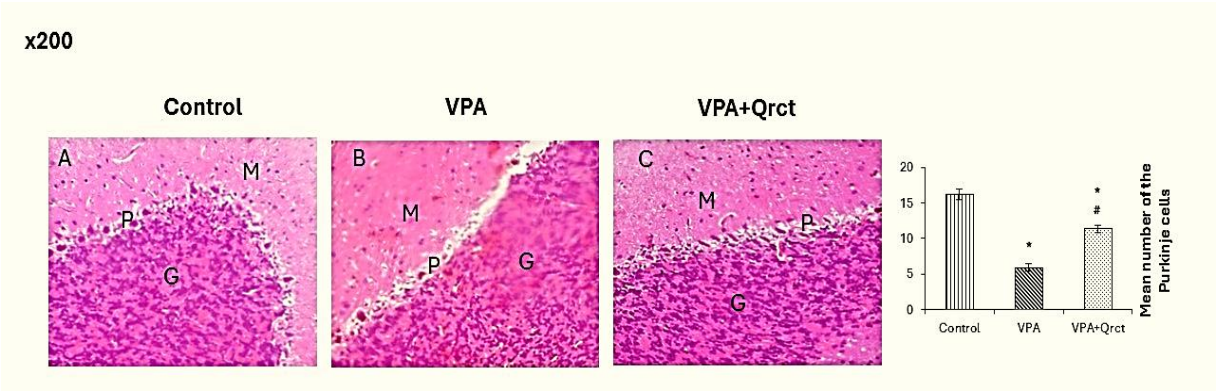

Fig. (1): H&E staining of rat cerebellar cortex. The cerebellar cortex of the control formed of outer molecular (M), middle Purkinje (P) and inner granular (G) that appeared with normal histological appearance. VPA group showed that Purkinje cells (P) appeared shrunken, disfigured with condensed chromatin and pyknotic nuclei. The granular layer (G) formed of densely packed cells. The molecular layer showed deeply stained pyknotic scattered cells. VPA+Qrct showed apparent amelioration of the previous mentioned three layers of the cerebellum specially the purkinje cell layer. x200 magnification.

Immunohistochemical results:

In the Bax stain, the VPA 's percentage area of Bax was dramatically higher than control (53.5 ±0.31 vs. 3.4±0.21, $p<0.05$). However, compared to the VPA, this percentage significantly decreased in the VPA+Qrct (13.2±0.04 vs. 53.5±0.31, $p<0.05$). (Fig 2.: A-D).

When compared to the control group, the VPA 's percentage area of NF-kB increased significantly (82.34 ±0.44 vs. 8.76±0.11, respectively, $p<0.05$) in the NF-kB stain. However, this percentage was dramatically lower in the VPA+Qrct than in the VPA (24.54±0.05 vs. 82.34 ±0.44, $p<0.05$). (Fig. 2: E-H).

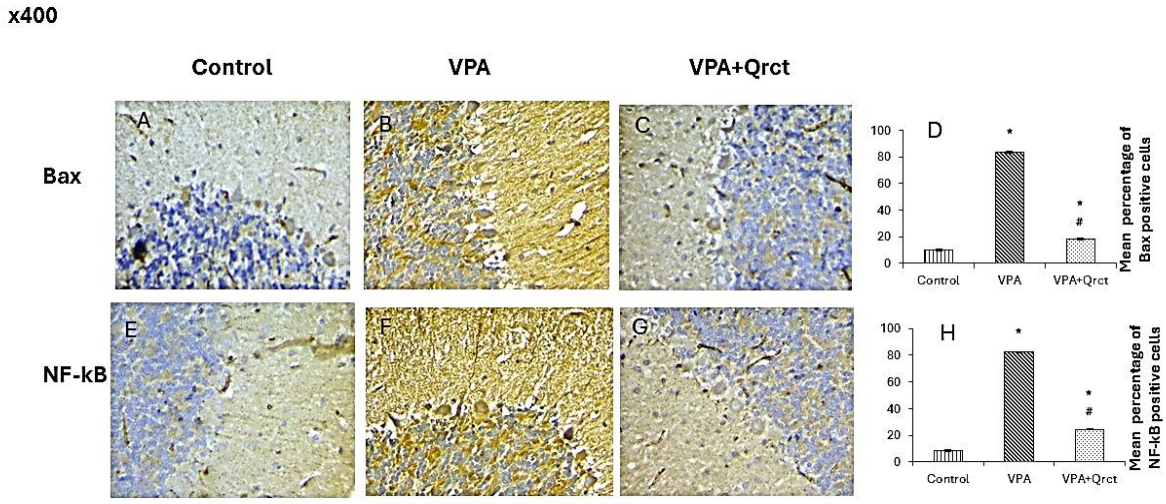

Fig (2): Representative micrographs of the different experimental groups showing a substantial upgrading of the Bax (A-D) and of the NF-kB (E-F) immunoreaction in the VPA and a significant declination of their percentage in the VPA +Qrct group. ×400 magnification.

Discussion

Care costs are predicted to rise steadily over the next ten years due to the rising prevalence of ASD. The goal of the recommended treatment for ASD is still to reduce symptoms. As a result, treating ASD through its pathogenic pathways should prioritize the use of neuroprotective medicines. Neuroprotective substances ought to preserve regular cellular activity and behavior while promoting brain regeneration (Molinari et al., 2025).

Consequently, this study illustrated how Qrct affects ASD. According to our findings, mice given VPA on the PND 14 displayed symptoms similar to autism, such as anxiety, social impairment, and compromised cognitive performance. Along with an inflammatory state and decreased BDNF level, the VPA group also shown a considerable disruption of the oxidative balance and brain enzyme function. The pathophysiology of ASD has also been linked to the up-regulation of TLR4 and NF-kB genes in the cerebellum. The favorable effect of Qrct on VPA-induced ASD was demonstrated by the amelioration of all VPA-induced alterations in the cerebellum tissue of autistic mice. A helpful model for examining the behavioral and

neurochemical alterations in autism in order to develop novel treatments is postnatal valproic acid. During the critical period of PND 14, the cerebellum experiences neuronal differentiation and synaptogenesis (Elgamal et al., 2023).

About 40% of autistic children meet the criteria for an anxiety diagnosis, which is regarded as a critical comorbidity in ASD (Farrag et al., 2024). When compared to the control, VPA exposure in the current study showed a dramatically increase in anxiety (reduced time spent in the open arm of EPM). Previous research has similarly demonstrated anxiogenic effects in rats exposed to VPA, which is consistent with our findings (Sailer et al., 2019).

In comparison to the control mice, VPA dramatically boosted rearing movements and decreased crossing slots and center entrance times in the open field test. These findings corroborated those of other research that found that administering VPA to the PND 14 caused cognitive impairment and autism-like characteristics, such as anxiety. VPA may alter gene transcription levels, potentially resulting in synaptic dysfunction and neurogenesis deficits (Gao et al., 2016).

One of the quantitative indicators used to diagnose ASD is social interaction. According to the results of the three-chambers test, the VPA group's social interaction behavior was significantly impaired. These findings were consistent with prior study that demonstrated a correlation between VPA exposure and a reduction in repetitive, stereotyped behaviors and socializing language (Larner et al., 2021). By inhibiting sodium-calcium channels, VPA may alter the neural circuits that cause ASD and reduce neuronal activity (Roullet et al., 2013). Moreover, VPA works by preventing histone deacetylase from altering DNA structure and influencing neuronal plasticity. This causes apoptotic cell death in a number of brain regions, especially the cerebellum, which impacts memory and behavior (Vecsey et al., 2007).

Using Qrct greatly improved the social and neurobehavioral alterations brought about by VPA adoption. This is consistent with earlier studies (Khan et al., 2024). According to a prospective study, children with ASD who received treatment with the same flavonoids for four months showed improvements in their attention and sociability, and a formulation containing Qrct significantly improved their adaptive functioning (Damasceno et al., 2025).

Compared to the control , the VPA 's biochemical profile revealed a substantial decrease in brain antioxidant SOD concentration and a notable increase in cerebellar MDA level. These results are consistent with earlier studies (Altun et al., 2018). According to prior research VPA raised cerebellar MDA, which was the consequence of a chain reaction involving harmful ROS and polyunsaturated fatty acids (Chauhan et al., 2011). Additionally, oxidative stress caused alterations in inflammation, the disintegration of proteins, lipids, and DNA, and brain tissue damage that could result in ASD (Fatemi et al., 2012).

However, compared to the VPA group, Qrct therapy increased SOD activity and lowered MDA, demonstrating their antioxidant action and supporting previous studies (de Mattos et al., 2020). Since the hydroxyl groups affixed to the aromatic rings possess the ability to neutralize reactive molecules like ROS, Qrct's antioxidant action is linked to its chemical composition. By chelating metals and/or absorbing free radicals, this substance can shield tissues from lipid peroxidation, which is mostly brought on by the superoxide anion and hydroxyl radical (de Mattos et al., 2020), in addition to its ability to up regulate of Nrf2/HO-1 signaling pathway (Li et al., 2019).

According to prevoius study VPA is a pro-inflammatory drug that may promote mitochondrial malfunction and/or neuroinflammation, both of which might result in ASD (Akintunde et al., 2015). Since VPA dramatically raised inflammatory markers (IL-6 and TNF-α) and lowered anti-inflammatory markers (IL-10) in the VPA group compared to the control mice, the current study supports the earlier findings. IL-6 offers a molecular marker to aid in early identification of ASD. Additionally, VPA-induced increases in IL-6 modify both excitatory and inhibitory synaptic forms, resulting in abnormal changes in synaptic transmission and dendritic spine length, shape, and distribution pattern (Wei et al., 2012).

Nonetheless, Qrct significantly reduced the inflammatory state caused by VPA, which is consistent with other research (Wu et al., 2019). Qrct may have an anti-inflammatory effect by reducing the production of inflammatory factors. Our findings demonstrated that Qrct had a neuroprotective effect by inhibiting the TLR4-mediated NF-κB pathway (Wu et al., 2019).

According to Wang et al. [23], the Qrct exhibited anti-inflammatory and anti-oxidative properties, possibly by inhibiting TLR4-mediated NF-κB, caspase-1, , TNF-α), IL-1β, and IL-6 (Wang et al., 2017).

It has been demonstrated that serotonin, a growth factor used in early brain development, negatively impacts social behavior (Alipour et al., 2022). Serotonin levels in the VPA group's cerebellum were lower. High levels of serotonin (5HT) in the whole blood during the early stages of brain development may account for this. It can enter the developing fetus's brain and cause the loss of 5-HT terminals through a negative feedback mechanism, which results in low intra-cerebral 5-HT concentrations in people with ASD. Throughout later development, this loss of 5-HT innervations continues. Consequently, autism symptoms manifest (Whitaker, 2004). However, in contrast to the VPA, Qrct was able to raise cerebellar serotonin. This outcome was in line with prior study (Abdallah et al., 2024).

Furthermore, BDNF affects synaptic plasticity by controlling BDNF levels for synapses. BDNF lowers neuroinflammation and oxidative stress, two factors that contribute to neurodegenerative diseases like ASD (Khan et al., 2024). A variety of neuropsychiatric and neurodevelopmental issues, including autistic disorders, have reportedly been linked to decreased BDNF levels (Bjørklund et al., 2020).

Cerebellar BDNF levels in the VPA were dramatically lower than those in the control in the current study. BDNF mRNA levels were decreased by VPA treatment (Fuentealba et al., 2019).

On the other hand, Qrct raised BDNF levels. These results aligned with previous studies (Khan et al., 2024). According to one study, Qrct has neuroprotective and anti-oxidative properties and increases BDNF levels by stimulating the BDNF pathway (Morimoto et al., 2023).

TLR4 expression is elevated in ASD patients' T cells in the central nervous system, and it facilitates inflammatory activation in a number of neurodegenerative illnesses. ROS production and the synthesis of pro-inflammatory cytokines are caused by the activation of the NF-κB pathway by TLR-4 signaling. It has also been demonstrated that ASD patients is associated with elevated NF-κB expression. By inducing NF-κB through subsequent gene transcription and protein synthesis, TLR4 activation activates microglia and results cytokine production and release (Farrag, et al., 2024).

In accordance with earlier research, our findings showed that the VPA group significantly increased TLR4/NF-kB cerebellar gene expression and cerebellar NF-kB immunoreaction when compared to the control (Farrag, et al., 2024).

In contrast, Qrct significantly reduced cerebellar NF-kB immunoreaction and TLR4/NF-kB gene expression in comparison to the VPA group, which is consistent with earlier study (Dong et al., 2018, Shams et al., 2022).

Oxidative stress is also a major factor in cell apoptosis, and earlier research in mice revealed that exposure to VPA led to increased expression of apoptotic markers that have been observed in the neuroepithelium and are thought to be the primary cause of altered embryonic signaling pathways and neural tube defects in the progeny (Taleb et al., 2021).

Therefore, we looked at the expression of the apoptotic marker Bax in the cerebellum in the current study. Our findings showed that the VPA group had an increased Bax cerebellar immunoreaction when compared to the control, which is consistent with other research (Sanaei et al., 2021). In contrast to VPA, Qrtc significantly reduced cerebellar Bax immunoreactivity, which is consistent with earlier study (Shams et al., 2022).

Conclusion

Through anti-oxidant, anti-inflammatory, neurotrophic, and antiapoptotic, as well as by down-regulating the cerebellar TLR4/NF-kB signaling pathway, Qrct ameliorated VPA-induced ASD.

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