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Quercetin-loaded nanophytosome ameliorates early life stress-induced hippocampal oxido-inflammatory damages

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ABSTRACT

Phytosome-based nanocarriers have emerged as innovative drug delivery systems in recent years, demonstrating significant potential in the treatment of neurodegenerative disorders. This study aimed to evaluate the therapeutic efficacy of quercetin-loaded nanophytosome (QNP) in modulating the oxido-inflammatory response in a rat model of early life stress (ELS) induced by maternal isolation (MI). To establish the ELS model, male rat pups were isolated from their dam for 3 hours daily from postnatal days 1–9. Following the lactation period (postpartum days 1–21), treatments with quercetin (10 and 40 mg/kg) and QNP (10 and 40 mg/kg) were administered continuously for 21 days. Cognitive behaviors, oxidative stress markers, hippocampal dopamine levels, and mRNA expression of TNF-α and IL-6 were assessed after ELS induction. Treatment with QNP (40 mg/kg) significantly improved cognitive function ($P < 0.01$), increased hippocampal dopamine levels ($P < 0.001$), and reduced oxidative stress ($P < 0.01$) as well as the expression of TNF-α ($P < 0.001$) and IL-6 ($P < 0.001$). In conclusion, QNP demonstrates potent hippocampal anti-oxidoinflammatory effects, making it a promising therapeutic candidate for mitigating the adverse effects of maternal isolation-induced early life stress.

1. Introduction

In recent years, flavonoids have garnered significant attention due to their diverse health benefits (Bo et al., 2024). As a primary source of natural antioxidants in the human diet, flavonoids, particularly quercetin, have become a focal point of scientific research (Shen et al., 2022). Quercetin, a prominent member of the flavonoid family, has attracted widespread interest for its multifaceted biological effects, including potent antioxidant and anti-inflammatory properties (Carrillo-Martinez et al., 2024; Lesjak et al., 2018). Despite these promising attributes, there remains a critical lack of clinically effective treatments to address functional impairments associated with neurodegenerative disorders (Cristino et al., 2020). Consequently, there is an urgent need to identify safe and effective therapeutic drug delivery systems for the treatment of neurodegenerative diseases. To address this challenge, the present study leverages phytosome technology as a promising solution.

Sharma et al. (2010) demonstrated that phytosomes exhibit superior performance compared to other vesicular drug delivery systems, such as liposomes and niosomes, in terms of the controlled release of phytoconstituents. This underscores their effectiveness as a delivery platform. Furthermore, the chemical bond formed between phospholipids and encapsulated phytochemicals ensures the structural integrity of the herbal components, making phytosomes an efficient delivery system for herbal extracts (Alharbi et al., 2021). Phytosomes enhance bioavailability, improve stability, facilitate gastrointestinal absorption, and amplify pharmacological activities, ultimately leading to a more effective drug product (Martins-Gomes et al., 2022).

Consistent with previous findings, this study highlights that QNP exhibits significant antioxidant effects comparable to those of free quercetin at equivalent dose levels (Abd El-Fattah et al., 2017).

Early life stress (ELS) arises from a variety of adverse experiences that activate stress-responsive physiological systems, depriving children of adequate social or physical care and exposing them to fear and anxiety (Herzberg and Gunnar, 2020). Additionally, different rearing environments during the postnatal period in rats have been shown to influence the expression of genes associated with inflammation (Ganguly and Brenhouse, 2015).

Stressors, such as maternal absence during this critical developmental phase, can lead to learning impairments (Xu et al., 2018). From a neurobiological perspective, one of the primary consequences of ELS is its impact on the hippocampus. As a key component of the brain, the hippocampus plays a vital role in memory consolidation (Derks et al.,

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2016).

Given that the characteristics of ELS may be attributed to or exacerbated by dysregulation of the dopamine system, investigating dopamine levels in relation to this disorder is crucial. Such an approach would offer valuable insights into the mechanisms underlying the impact of ELS on dopamine signaling (Rodrigues et al., 2011; Smith and Pollak, 2020). Additionally, stress has been shown to promote inflammatory responses in the hippocampus (Black, 2002). Despite these findings, there remains a lack of effective treatments to improve functional outcomes associated with neurodegenerative disorders (Cui et al., 2022). Exposure to stress during critical developmental periods, such as maternal care deprivation, can have profound and lasting effects on brain function, cognitive behaviors, metabolism, and oxidative-inflammatory markers (Ganguly and Brenhouse, 2015; Morelli et al., 2021; Naninck et al., 2015).

Interleukin-6 (IL-6) plays a multifaceted role in the stress response, serving as a key link between the immune and neuroendocrine systems. Chronic stress exposure has been shown to induce IL-6-mediated plasticity in the hypothalamic-pituitary-adrenal (HPA) axis. Furthermore, dysregulation of IL-6 has been consistently reported in individuals with ELS experiences. Growing evidence suggests that IL-6 dysregulation may serve as a potential biomarker for stress-related disorders, highlighting its significance in understanding the long-term consequences of ELS (Agorastos et al., 2019).

Previous studies have demonstrated that adverse events during early life increase the risk of neurodevelopmental disorders in adulthood (Kundakovic and Jaric, 2017). Maternal isolation (MI) is a well-established animal model used to investigate the mechanisms through which early environmental stress influences biological injury in the developing brain (Mumtaz et al., 2018; Ou-Yang et al., 2022). The psychopathological trajectory associated with ELS may be influenced by the type of stress, the developmental period during which it occurs, as well as environmental and protective factors (Heim and Binder, 2012). In summary, the present study aims to explore the potential of QNP to enhance dopaminergic system function, modulate oxidative stress mechanisms, and regulate mRNA expression of TNF-α and IL-6 in the hippocampus of an MI rat model through physiological and molecular investigations.

2. Material and method

2.1. Synthesis of quercetin nanophytosomes

To achieve this objective, we synthesized quercetin nanophytosomes (QNP) in our previous study. Briefly, the synthesis involved dissolving 6 g of quercetin, 3 g of phosphatidylcholine, and 2.16 g of Tween 80 in 12 ml of warm ethanol (55°C). Subsequently, 120 ml of 96 % ethanol was added, followed by the incorporation of phosphate buffer solution. In addition, scanning electron microscopy (SEM) analysis was performed to assess the average vesicular size and morphological characteristics of the QNP (Moghaddam et al., 2023).

2.2. Animal and induction of early life stress

All experiments were conducted in compliance with the ethical guidelines approved by the University of Mazandaran Committee under protocol number (IR.UMZ.REC.1400.046) and in accordance with current national regulations. Female and male Wistar rats, obtained from the Animal Sciences Center at the University of Mazandaran, were housed in pairs in plastic cages under controlled conditions: a temperature of 25 ± 3°C and a 12/12-hour light/dark cycle for mating purposes. The day of delivery was designated as postnatal day 0 (PND0). ELS was induced by separating the pups from the dam from PND1 to PND9 for 3 hours daily (9:00–12:00 AM). During this period, the pups were kept individually in small glass containers (20 × 20 × 5 cm) without bedding material at a temperature of 30–32°C. Half of the pups were assigned to the ELS group, while the control and positive control groups remained undisturbed with their mothers. The ELS experimental groups were randomly divided into five groups: ELS (pups exposed to ELS only), ELS-Q10 (pups exposed to ELS and treated with quercetin at 10 mg/kg), ELS-Q40 (pups exposed to ELS and treated with quercetin at 40 mg/kg), ELS-QNP10 (pups exposed to ELS and treated with QNP at 10 mg/kg), and ELS-QNP40 (pups exposed to ELS and treated with QNP at 40 mg/kg).The control group was given only saline by oral gavage for 21 days starting from PND21, whereas the positive control group received QNP 40 mg/kg by oral gavage during the same period (Fig. 1).

The rats were acclimatized for one week in the animal center prior to mating to allow them to adapt to their new environment (under a 12/12-hour light/dark cycle). Subsequently, male and female rats were randomly paired in cages (25 × 40 × 12 cm) and allowed to mate for four days. The offspring from these pairings were used as experimental subjects. Twenty-four hours after birth, the pups were randomly assigned to either the control or MI groups.

2.3. Novel object test

The novel object recognition test was conducted in three phases: habituation, familiarization, and memory testing. During the habituation phase, rats were allowed to freely explore the empty testing box for 5 minutes without any objects present. Twenty-four hours after the habituation phase, the familiarization trial was performed by placing the rat in the testing arena containing two identical objects (A, A). The animal was positioned at the center of the arena and allowed to explore for 5 minutes. In the final phase, one of the familiar objects (A) was replaced with a novel object (B). Each rat was released into the arena for 5 minutes and allowed to freely explore the objects (A and B). The discrimination index (DI) was calculated as follows: (time spent exploring the novel object / total time spent exploring both objects) × 100.

2.4. Tissue and homogenate preparation

Following the behavioral tests, 42-day-old rats were anesthetized and decapitated. The hippocampus tissue was then carefully dissected for biochemical analysis and gene expression studies. Hippocampal tissue samples (150–200 mg) from each subject were homogenized in 1 ml of a buffer solution (0.32 mol/l sucrose, 1 mmol/l EDTA, 10 nmol/l Tris-HCl, pH = 7.4). The homogenate was centrifuged at 13,600 rpm and 4°C for 30 minutes. The resulting supernatant was collected and stored at −80°C until further analysis.

2.5. Measurements of antioxidant enzymes activities

The activity of CAT and SOD in the hippocampus was measured following the method described by Genet et al. (2002). Briefly, for CAT activity, 20 µl of the supernatant was mixed with 50 mM sodium phosphate buffer (pH=7) and 10 mM hydrogen peroxide. The decrease in absorbance was monitored at 240 nm for 5 minutes at 25°C against a blank. CAT activity was expressed as the percentage of H_2O_2 consumed/minute/milligram of protein. For SOD activity, 20 µl of the supernatant was combined with 0.1 mM EDTA, 0.48 mM pyrogallol, and 50 mM sodium phosphate buffer (pH=7). The absorbance was recorded spectrophotometrically at 420 nm (25°C) for 3 minutes. One unit of SOD activity was defined as the amount of enzyme required to inhibit the autoxidation of pyrogallol by 50 %.

2.6. Estimation of lipid peroxidation

Lipid peroxidation products were quantified using the method described by Esterbauer and Cheeseman (1990). Briefly, 1 ml of hippocampus tissue homogenate was mixed with 1 ml of 0.67 % thiobarbituric acid and 0.5 ml of 20 % trichloroacetic acid. The mixture was

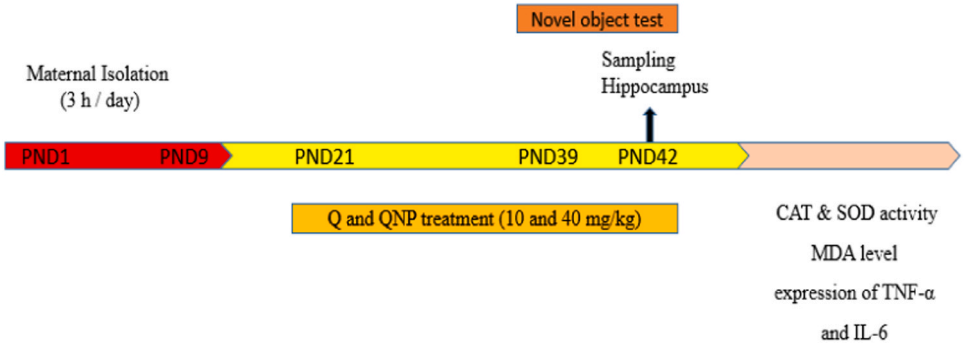

Fig. 1. Timeline of the experimental design.

incubated in a boiling water bath for 45 minutes. After cooling, the samples were centrifuged to remove the precipitate. The absorbance of the supernatant was then measured at a wavelength of 535 nm.

2.7. Measurement of protein content

For the calculation of the protein concentrations of the homogenates of hippocampus samples, the Bradford procedure was employed (Bradford, 1976). Bovine serum albumin was applied as the standard in this experiment.

2.8. Measurements of dopamine assay

The dopamine levels in the hippocampus were measured using the method described by Guo et al. (2009). Briefly, hippocampal tissue was homogenized in 1 ml of ice-cold buffer containing 50 mM Tris–HCl, 0.32 M sucrose, and 1 mM EDTA, adjusted to a final volume of 1 ml, and maintained at 30°C. The homogenate was then centrifuged at 12, 000 rpm for 30 minutes. To quantify dopamine levels, 1 ml of the hippocampal tissue homogenate was mixed with 1 ml of ferric chloride and 1 ml of potassium ferricyanide. The mixture was diluted to 25 ml with distilled water and allowed to stand at room temperature for 30 minutes. Absorbance was measured at a wavelength of 735 nm, and the results were expressed as ng of dopamine per mg of protein.

2.9. Measurement of mRNA expression by quantitative reverse transcription-PCR (qRT-PCR)

RNA samples were extracted from hippocampal tissue homogenates using the RNeasy purification kit (QIAGEN) in accordance with the manufacturer's instructions. The concentration and purity of the RNA were determined spectrophotometrically by measuring absorbance at 260 nm and 280 nm. To eliminate genomic DNA contamination, 1 μg of total RNA was treated with RNase-free DNase I (Fermentas) following the manufacturer's protocol. First-strand cDNA synthesis was performed using Superscript III Reverse Transcriptase (Fermentas) as outlined in the manufacturer's guidelines. The primer sequences used for qRT-PCR are listed in Table 1. cDNA samples were analyzed by Real-Time PCR using the SYBR Green kit (TaKaRa). Gene expression levels were quantified based on CT values and calculated using the $2^{-\Delta\Delta CT}$ method.

Table 1
Sequences of primers used in qRT-PCR.

Gene	Primer	Sequence	Amplicon lengths (bp)
GAPDH	forward	5´-ATCCTGCACCACCAACTG C−3´	129
	reverse	5´-ACGCCACAGCTTTCCAGAG−3´	
TNF-α	forward	5′-GGAGGAGCAGCTGGAGTG−3′	131
	reverse	5′-CCTTGAAGAGAACCTGGGAGTAGA−3′	
IL−6	forward	5′-TCACAGAGGATACCACCCACAA−3	146
	reverse	5′-CAGTGCATCATCGCTGTTCATAC−3′	

2.10. Statistical analysis

Since the data exhibited normal distribution and homogeneity of variance, one-way ANOVA was used to compare the effects of different doses of quercetin and QNP with the control group. Statistical differences among the groups were assessed using one-way ANOVA, followed by post-hoc analysis with the Tukey HSD test to evaluate specific group comparisons. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of QNP on recognition memory consolidation in the NORT

As illustrated in Fig. 2, one-way ANOVA analysis indicated that ELS significantly reduced the discrimination index [F (6, 28) = 12.2, $P < 0.001$]. Post hoc Tukey's test demonstrated that treatment with QNP at both 10 mg/kg and 40 mg/kg significantly improved the discrimination index in the ELS group ($P < 0.01$). Notably, the high dose of QNP (40 mg/kg) was more effective ($P < 0.05$) in increasing the time spent exploring the novel object compared to the familiar object, as well as in comparison to the equivalent dose of free quercetin (40 mg/kg).

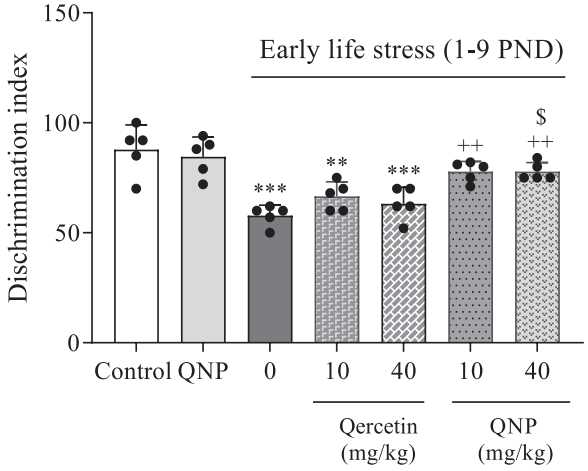

Fig. 2. Effects of QNP treatments on recognition memory in early life stress induced by maternal isolation. The experiment was applied to six male rats and data are displayed as means ± SD. QNP; quercetin-loaded nanophytosome. $^{***}P < 0.001$, $^{**}P < 0.01$ as compared to control group. $^{++}P < 0.01$ as compared to 0. $^{\$}P < 0.05$ as compared to quercetin 40.

3.2. Effect of QNP on hippocampal CAT activity in ELS

Repeated exposure to ELS significantly affects hippocampal CAT enzyme activity. As depicted in Fig. 3, ELS induction led to a marked reduction in CAT activity in the hippocampus compared to the control group [F (6, 21) = 9.269, $P < 0.01$]. However, treatment with QNP at 40 mg/kg significantly increased hippocampal CAT activity relative to the ELS group ($P < 0.01$). Furthermore, QNP (40 mg/kg) demonstrated greater efficacy in enhancing hippocampal CAT activity ($P < 0.05$).

3.3. Effect of QNP on hippocampal SOD activity in ELS

The results of the statistical analysis showed that ELS exposure reduces hippocampal SOD activity as compared to the control group [F (6, 21) = 19.70, ($P < 0.001$)]. Compared to the ELS group, QNP (10 and 40 mg/kg) showed higher SOD activity ($P < 0.01$). Also, treatment with QNP (40 mg/kg) revealed more sufficiency to enhance the hippocampal SOD activity ($P < 0.05$) (Fig. 4).

3.4. Effect of QNP on hippocampal MDA level in ELS

MDA levels were measured to assess the potential role of oxidative stress in the hippocampus following exposure to ELS. Rats subjected to ELS exhibited a significant increase in hippocampal MDA levels compared to the control group [F (6, 21) = 18.06, $P < 0.001$]. Quantitative analysis revealed that treatment with QNP (40 mg/kg) significantly reduced hippocampal MDA levels in ELS-exposed rats compared to the untreated ELS group ($P < 0.01$). Furthermore, QNP (40 mg/kg) demonstrated a greater ability to lower hippocampal MDA levels compared to an equivalent dose of free quercetin (40 mg/kg) ($P < 0.001$) (Fig. 5).

3.5. Effect of QNP on hippocampal dopamine level in ELS

As shown in Fig. 6, one-way ANOVA analyses showed that the ELS had a significant reduction in the hippocampal dopamine level [F (6, 21) = 18.99, ($P < 0.001$)]. Quantitative analysis exhibited that the hippocampal dopamine level was significantly increased in the ELS rats following the administration of QNP (40 mg/kg) as compared with the ELS rats ($P < 0.001$). Importantly, treatment with QNP (40 mg/kg) showed more efficiency to elevate the hippocampal dopamine level as compared with quercetin (40 mg/kg) ($P < 0.001$).

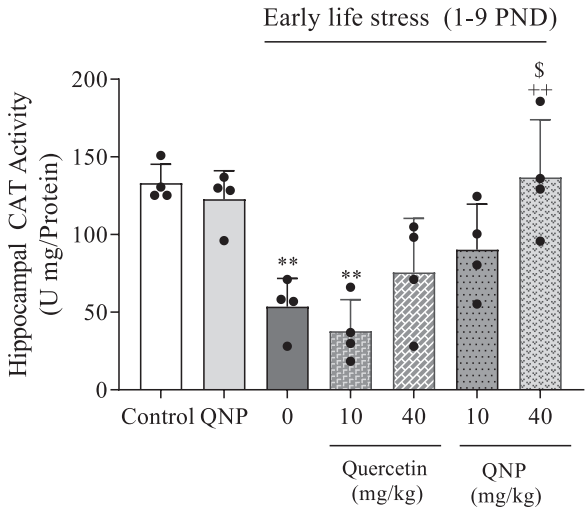

Fig. 3. Effects of QNP treatments on catalase activity in early life stress induced by maternal isolation. The experiment was applied to six male rats and data are displayed as means ± SD. QNP; quercetin-loaded nanophytosome. $^{**}P < 0.01$ as compared to control group. $^{++}P < 0.01$ as compared to 0. $^{\$}P < 0.05$ as compared to quercetin 40.

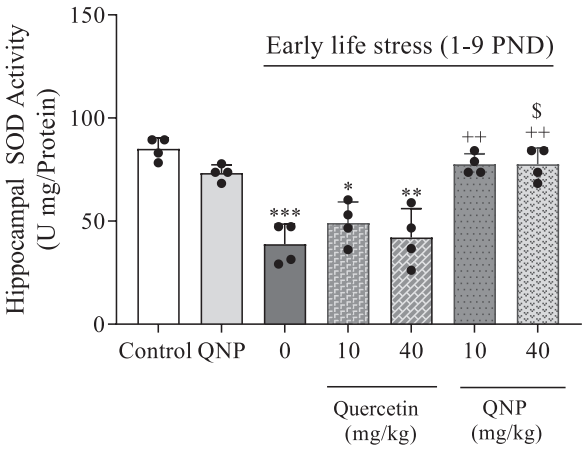

Fig. 4. Effects of QNP treatments on SOD activity in early life stress induced by maternal isolation. The experiment was applied to six male rats and data are displayed as means ± SD. QNP; quercetin-loaded nanophytosome. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.005$ as compared to control group. $^{++}P < 0.01$ as compared to 0. $^{\$}P < 0.05$ as compared to quercetin 40.

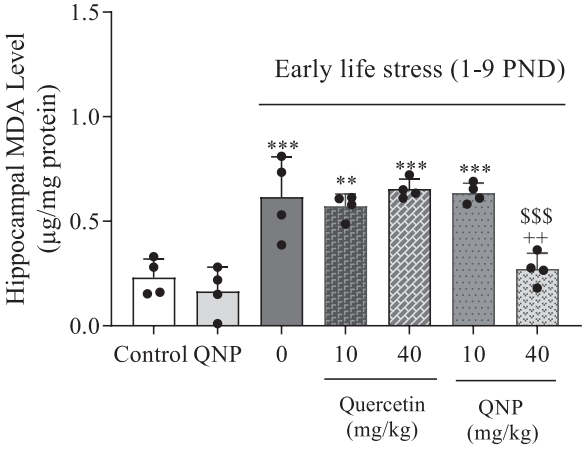

Fig. 5. Effects of QNP treatments on MDA level in early life stress induced by maternal isolation. The experiment was applied to six male rats and data are displayed as means ± SD. QNP; quercetin-loaded nanophytosome. $^{***}P < 0.001$, $^{**}P < 0.01$ as compared to control group. $^{++}P < 0.01$ as compared to 0. $^{\$\$\$}P < 0.001$ as compared to quercetin 40.

3.6. Effect of QNP on the expression of TNF-α and IL-6 in the hippocampal area of ELS

TNF-α plays a critical role in initiating and regulating immune and inflammatory responses. Recognized as a potent proinflammatory molecule, TNF-α exerts stimulatory effects on various immune cells, underscoring its importance as a key regulator of immune function and inflammation. Analyses were conducted to evaluate the expression of inflammatory factors in the hippocampus of rats exposed to early life stress (ELS). The expression of TNF-α in the hippocampus of ELS rats was significantly elevated compared to the control group [F (6, 21) = 101.7, ($P < 0.001$)]. Quantitative analysis revealed that TNF-α expression was significantly reduced in ELS rats following treatment with quercetin (40 mg/kg) and QNP (10 and 40 mg/kg) compared to untreated ELS rats ($P < 0.001$). Notably, QNP (10 and 40 mg/kg) demonstrated more efficacy in reducing TNF-α expression in the hippocampus compared to equivalent doses of free quercetin (10 and 40 mg/kg) ($P < 0.001$) (Fig. 7).

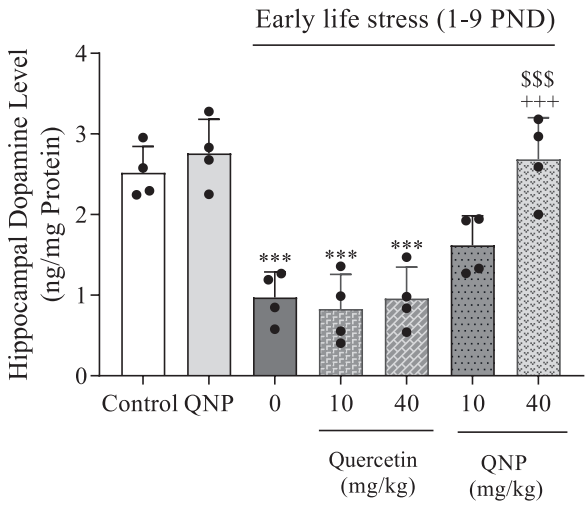

Fig. 6. Effects of QNP treatments on dopamine level in early life stress induced by maternal isolation. The experiment was applied to six male rats and data are displayed as means ± SD. QNP; quercetin-loaded nanophytosome. $^{***}P < 0.001$ as compared to control group. $^{+++}P < 0.001$ as compared to 0. $^{\$\$\$}P < 0.001$ as compared to quercetin 40.

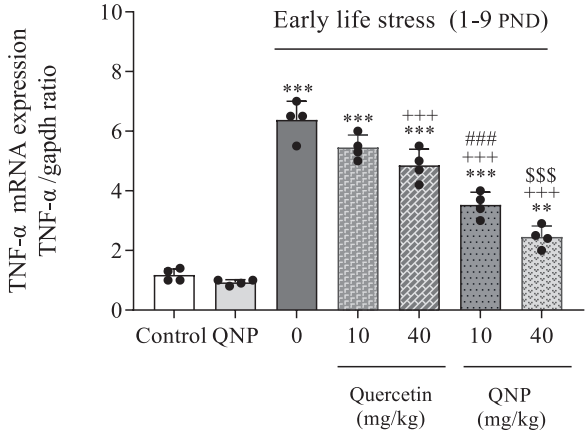

Fig. 7. Effects of QNP treatments on the expression of TNF-α in early life stress induced by maternal isolation. The experiment was applied to six male rats and data are displayed as means ± SD. QNP; quercetin-loaded nanophytosome. $^{***}P < 0.001$, $^{**}P < 0.01$ as compared to control group. $^{+++}P < 0.001$ as compared to 0. $^{\#\#\#}P < 0.001$ as compared to quercetin 10. $^{\$\$\$}P < 0.001$ as compared to quercetin 40.

Both classical and trans-signaling pathways involving IL-6 play a significant role in neurodegenerative diseases, with dysregulation of IL-6 being a critical factor in the pathology of various neurological disorders. Therefore, understanding IL-6 signaling is essential for advancing our study. As shown in Fig. 8, the expression of IL-6 in the hippocampus was significantly higher in ELS rats compared to the control group [$F(6, 21) = 163.9$, $P < 0.001$]. Additionally, one-way ANOVA analysis revealed that IL-6 expression in the hippocampus was significantly reduced following treatment with quercetin (10 and 40 mg/kg) and QNP (10 and 40 mg/kg) compared to untreated ELS rats ($P < 0.001$). Importantly, our data demonstrate that QNP (10 and 40 mg/kg) was more effective in reducing IL-6 expression in the hippocampus compared to equivalent doses of free quercetin (10 and 40 mg/kg) [$P < 0.05$ and $P < 0.001$, respectively].

4. Discussion

The present study aimed to evaluate the efficacy of QNP as a

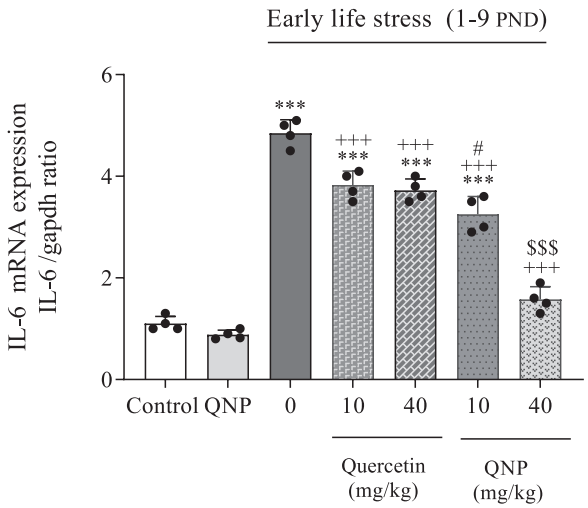

Fig. 8. Effects of QNP treatments on the expression of IL-6 in early life stress induced by maternal isolation. The experiment was applied to six male rats and data are displayed as means ± SD. QNP; quercetin-loaded nanophytosome. $^{***}P < 0.001$ as compared to control group. $^{+++}P < 0.001$ as compared to 0. $^{\#}P < 0.05$ as compared to quercetin 10. $^{\$\$\$}P < 0.001$ as compared to quercetin 40.

potential therapeutic approach to ELS-induced oxido-inflammatory stress in the hippocampus of male rats. The findings demonstrated that ELS led to cognitive impairment, oxidative damage, and elevated hippocampal expression of TNF-α and IL-6. Additionally, the data indicated that ELS significantly altered dopamine levels in the hippocampus.

Plant-based products represent a significant sector of pharmaceuticals, many of which consist of water-soluble or polar molecules. However, due to their molecular size and low solubility, these compounds often cannot passively diffuse across the blood-brain barrier, limiting their bioavailability and therapeutic efficacy (Reddy et al., 2021; Anand et al., 2022). Consequently, phytosome technology has emerged as a promising approach to enhance the biological activity and bioavailability of these compounds compared to free herbal extracts. Extensive research suggests that phytosomes, as a novel drug delivery system, offer an optimal formulation for improving oral bioavailability (Barani et al., 2021; Abd El-Fattah et al., 2017). Additionally, evidence indicates that the particle size of formulated nanoparticles can enhance absorption, enable passive drug targeting, and improve drug-loading capacity (Gelperina et al., 2005; Dang and Guan, 2020).

In this study, the therapeutic efficacy of QNP was evaluated in rats using an experimental model of ELS induced by MI. The findings indicate that the developing brain is particularly vulnerable to environmental factors during the early postnatal period, which can significantly influence behavioral outcomes and the functional organization of the brain (Sasagawa et al., 2017). The MI paradigm has been widely employed in rodent studies to assess the effects of ELS on behavioral and biochemical markers, as well as changes in the hippocampus, which are closely linked to the function of the dopaminergic system (Liao et al., 2019; Richardson et al., 2021).

Early life adversities, as previously documented, exert long-lasting behavioral and neurochemical effects on individuals. Given the critical role of the infant-mother bond in the maturation of the offspring's neurochemical system, MI as a stress-inducing process significantly increases the risk of cognitive disorders in adulthood (Amini-Khoei et al., 2017). Consistent with prior studies, the present study demonstrated that MI adversely affects the hippocampus and behavior of rats, leading to cognitive dysfunction, altered antioxidant enzyme activities, dysregulated gene expression, and disruptions in the dopaminergic system (Schiavone et al., 2015; Nishi, 2020; Amini-Khoei et al., 2019). Furthermore, the novel object recognition test (NORT) revealed that ELS

induction resulted in cognitive deficits. In alignment with earlier findings, the results of this study indicate that quercetin ameliorated cognitive deficits and hippocampal impairment in rats exposed to chronic stress (McLeod et al., 2001; Mehta et al., 2017).

Administration of QNP significantly improved cognitive performance, whereas free quercetin exhibited weaker effects. Reduced hippocampal neuroplasticity has been strongly linked to depression in animal models, with dopamine playing a critical role in stress-associated depression by influencing neurogenesis and development in the hippocampus (Serafini et al., 2014). Furthermore, ELS has been shown to exert broad effects on the development of the dopaminergic system across various brain regions during critical periods of brain maturation (Bonapersona et al., 2018). Consistent with previous evidence, our findings indicate that MI leads to a reduction in dopamine levels (Kawakami et al., 2013). Dopamine is well-documented to play a pivotal role in regulating mood and supporting cognitive functions (Gulpinar and Yegen, 2004). Previous studies have also highlighted the ability of quercetin to enhance hippocampal dopamine levels, thereby improving cognitive performance (Sarubbo et al., 2018). Given the established positive correlation between elevated ROS production and oxidative stress following ELS (Amini-Khoei et al., 2017), it is plausible that QNP ameliorates cognitive impairments and enhances dopaminergic function by mitigating oxidative stress. In light of the critical role of neural oxidative stress in the pathophysiology of ELS, this study evaluated CAT and SOD activities, as well as MDA levels, in the hippocampus. The findings revealed that chronic MI-induced stress was associated with increased ROS and MDA levels. The protective effects of quercetin in the ELS model have been demonstrated in prior studies. For instance, Serena Silvestro et al. reported quercetin's ability to inhibit oxidative stress induced by maternal separation (Silvestro et al., 2021).

Regarding its antioxidant properties, QNP treatment significantly reduced hippocampal MDA levels in the ELS model. SOD and CAT are well-known antioxidant enzymes that enhance the sensitivity of defense mechanisms against oxygen radicals (Shen et al., 2022). This study demonstrated that MI could diminish the activity of SOD and CAT. As an antioxidant compound, quercetin inherently possesses the ability to efficiently scavenge excess oxidants, thereby regulating enzyme activity. Notably, treatment with QNP effectively prevented the MI-induced reduction in SOD and CAT activities. The observed increase in SOD and CAT activities may be attributed to the upregulation of their gene expression following quercetin treatment.

The regulatory effect of quercetin on genes associated with the expression of antioxidant enzymes has been well-documented in previous studies (Kampkötter et al., 2008; Singh et al., 2022). In addition to oxidative stress, inflammation plays a significant role as a pathological factor in ELS, contributing to cell death (Liao et al., 2019). Activated microglia can influence neural activity by increasing the production of inflammatory mediators, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (Wu et al., 2017). There is substantial evidence that neuroglial activation and neuroinflammatory responses may contribute to neural dysfunction in maternal isolation (MI)-induced early life stress (Banqueri et al., 2019).

Moreover, previous research by Wang et al. (2020) has shown that maternal separation upregulates pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, in both the prefrontal cortex (PFC) and hippocampus. This reinforces the idea that chronic stress can trigger an inflammatory response in these brain regions, potentially contributing to the cognitive impairments observed in our study. Our findings reveal that QNP significantly reduced the levels of pro-inflammatory cytokines, IL-6 and TNF-α, in the hippocampus. These anti-inflammatory effects likely play a key role in the observed improvement in cognitive function following QNP treatment, highlighting its promising therapeutic potential for stress-related disorders, particularly those involving hippocampal dysfunction.

5. Conclusion

In summary, this study highlights the potential therapeutic benefits of QNP in targeting oxidative stress and inflammation in the hippocampus. The findings suggest that QNP administration effectively reduces oxidative stress and inflammatory responses, which may lead to improved cognitive behavior in an ELS model. These results underscore the promise of QNP as a therapeutic intervention for stress-related cognitive impairments.

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CRediT authorship contribution statement

Jelodar Sedigheh khanjani: Writing – review & editing, Methodology, Formal analysis. **Hajizadeh Moghaddam Akbar:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Ranjbar Mojtaba:** Writing – review & editing, Formal analysis. **Eslami Ali:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

The authors involved in this research do not have any financial ties or conflicts of interest with any industries or parties.

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