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Neurobehavioral and metabolic effects of polyphenol-rich olive oil in male rats during the adolescent-to-young adult transition: a comparative study

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Abstract

Adolescence and the transition to young adulthood represent critical periods for brain development, marked by increased susceptibility to neuroinflammation and metabolic disturbances. Olive oil polyphenols are known for their antioxidant and anti-inflammatory properties, yet their specific effects on neurobehavioral outcomes during this developmental window remain unclear. This study aimed to compare the effects of high-polyphenol olive oil (HPOO) and low-polyphenol olive oil (LPOO) on neuroinflammatory markers, lipid profiles, cognitive performance, and emotional behaviors in male Sprague Dawley rats aged 6–14 weeks. Twenty-four animals were randomly assigned to control, LPOO, or HPOO groups and received oral gavage for 8 weeks. Behavioral assessments included the Morris water maze (MWM), open-field test (OFT), elevated plus maze (EPM), and forced swim test (FST). Serum triglycerides, LDL/HDL ratio, and cytokine levels in the hippocampus and prefrontal cortex were evaluated via ELISA. HPOO significantly reduced triglyceride levels and LDL/HDL ratio, while LPOO lowered only triglycerides. HPOO also decreased the TNF-α/IL-10 ratio in both brain regions, suggesting reduced neuroinflammation, whereas LPOO showed effects limited to the prefrontal cortex. Elevated TG and TNF-α/IL-10 levels were positively correlated with anxiety-like behaviors and inversely related to spatial memory performance. These findings indicate that HPOO has superior modulatory effects on neuroinflammatory and metabolic parameters compared to LPOO. Regular consumption of HPOO may support neurodevelopmental health during adolescence and early adulthood by reducing inflammation and improving lipid balance, potentially contributing to improved emotional and cognitive outcomes.

Keywords Polyphenols · Olive oil · Adolescence · Neuroinflammation · Lipid profile · Behavior

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Introduction

Adolescence represents a critical neurodevelopmental window marked by extensive neurobiological, cognitive, and behavioral transformations [4, 23]. During this period, the brain undergoes substantial structural and functional remodeling, particularly in regions such as the prefrontal cortex (PFC) and hippocampus (HC), which are integral to executive functioning, emotional regulation, and memory formation [1, 10]. This heightened neuroplasticity renders the adolescent brain, especially sensitive to environmental stressors, including neuroinflammatory challenges [2, 6]. Accumulating evidence suggests that neuroinflammation during adolescence can induce long-lasting impairments in cognition and affective behavior [14, 22, 26].

In rodents, 6–8 weeks of age corresponds to adolescence, while 10–15 weeks reflects the transition into young adulthood [20]. Therefore, the present study, which spanned 6–14 weeks of age, covers the critical adolescent-to-young adult period. This clarification is important for the accurate translational interpretation of our findings.

Neuroinflammatory processes are mediated by a dynamic balance between pro- and anti-inflammatory cytokines. Among these, tumor necrosis factor-alpha (TNF-α) has been associated with hippocampal synaptic dysfunction, neuronal apoptosis, and impaired memory formation [9, 27, 28]. Elevated TNF-α levels have also been linked to depression-like phenotypes in both animal models and human clinical studies [11, 15, 16, 25]. Conversely, anti-inflammatory cytokines such as interleukin-10 (IL-10) act as endogenous neuroprotective agents by counterbalancing pro-inflammatory signaling and mitigating associated behavioral and cognitive deficits [12]. The TNF-α/IL-10 ratio has thus emerged as a key indicator of neuroinflammatory status and a potential predictor of neurobehavioral outcomes [29].

The Mediterranean diet (MD), known for its neuroprotective properties, has been associated with reduced incidence of cognitive decline and affective disorders [24]. Olive oil, the principal fat source in this diet, comprises mainly triacylglycerols and over 230 minor compounds, including polyphenolic antioxidants such as oleuropein, hydroxytyrosol, and flavonoids [3]. The phenolic content of olive oil varies significantly based on agricultural practices, extraction methods, and storage conditions. It is available in two main forms: unrefined extra-virgin olive oil (EVOO), rich in polyphenols, and refined olive oil, which undergoes processing that reduces its antioxidant content [7].

While the peripheral health benefits of olive oil are well-established, growing evidence suggests its polyphenolic components may also confer neuroprotective effects. Preclinical studies have shown that EVOO supplementation improves cognitive function, reduces anxiety and depression-like behaviors, and attenuates neuroinflammation [18, 21]. However, most of this research has been conducted in aged or disease-model animals [8, 21], with limited attention given to adolescence—a period of both neurodevelopmental vulnerability and opportunity.

Previous studies have largely investigated the effects of extra-virgin olive oil (EVOO) in aged animals or disease models, reporting improvements in memory, reduction in neuroinflammation, and modulation of lipid metabolism. However, little is known about whether these benefits extend to the adolescent-to-young adult developmental window, a critical period for long-term brain health. Moreover, no prior study has directly compared high- versus low-polyphenol olive oils in this context. Therefore, the present study is novel in assessing the differential neurobehavioral, metabolic, and inflammatory outcomes of HPOO and LPOO in male rats during this transitional stage.

Materials and methods

Animals

Male Sprague–Dawley rats, 6 weeks of age, were housed under standardized laboratory conditions: a controlled ambient temperature of 22 ± 1 °C, relative humidity maintained at 60%, and a 12-h light/dark cycle. Throughout the experiment, the animals had unrestricted access to food and water. All procedures were conducted in accordance with ethical guidelines and were approved by the Animal Care and Use Committee of Dokuz Eylul University, Faculty of Medicine (approval no: 20/2017).

Preparation of olive oil formulations

High-polyphenol and low-polyphenol olive oils were supplied by TUAY Co. Ltd. (Turgut Anadolu Yatirim). Memecik variety olives (Olea europaea) were cold-pressed (≤ 27 °C) within 2–4 h of harvesting using a proprietary extraction method designed to enhance polyphenol retention. The oils were stored at 18–22 °C until use. Quantification of total polyphenol content was performed via high-performance liquid chromatography (HPLC) and confirmed by the Ministry of Agriculture and Forestry's Aydın Special Food Control Laboratory, revealing concentrations of 775 mg/kg in HPOO and 77 mg/kg in LPOO.

Experimental design

Animals were randomly divided into three groups ($n = 8$ per group) and received daily oral gavage for 8 weeks as follows: control (1 mL distilled water), LPOO (1 mL/day), and HPOO (1 mL/day). All groups were fed a standard chow diet containing 13% fat, 25% protein, and 62% carbohydrates. Body weight was recorded weekly. Behavioral assessments for learning, memory, anxiety, and depression were conducted using the Morris water maze (MWM), elevated plus maze (EPM), open field test (OFT), and forced swim test (FST). Following behavioral evaluations, animals were anesthetized with CO_2 and sacrificed by cardiac puncture for blood and brain tissue collection. The hippocampus and prefrontal cortex were carefully dissected and stored at − 80 °C for biochemical analysis.

Biochemical analysis

Plasma levels of triglycerides (TG), total cholesterol (TC), HDL, LDL, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using a Beckman Coulter AU680 autoanalyzer. Results were expressed in mg/dL for lipid markers and U/L for liver enzymes. Cytokine levels (TNF-α and IL-10) in the HC and PFC were quantified using commercial ELISA kits (Bioassay Technology Laboratory, Shanghai, China) according to the manufacturer's protocols.

Behavioral testing

Morris water maze (MWM)

The spatial learning and memory abilities were assessed using a circular pool (140 cm diameter, 75 cm height) filled with opaque water to a depth of 50 cm. A submerged platform was hidden 1 cm below the water surface. Rats underwent 4 days of acquisition trials, followed by a probe trial on day five where the platform was removed. Behavioral parameters, including latency to locate the platform and time spent in target and opposite quadrants, were recorded using the Noldus EthoVision tracking system.

Open field test (OFT)

OFT was performed in a 1×1 m square arena surrounded by 50 cm high opaque walls. Each rat was placed at the center, and activity was recorded for 5 min using an overhead camera. Parameters such as total distance moved and time spent in central vs. peripheral zones were analyzed to assess anxiety-like behavior and locomotion.

Elevated plus maze (EPM)

The EPM apparatus consisted of two open arms and two enclosed arms (50 cm length, 10 cm width), elevated 50 cm above the ground, with a central platform (5×5 cm). Rats were placed on the central platform facing an open arm and

allowed to explore for 5 min. The time spent in, and entries into, open and closed arms were recorded to assess anxiety-like behavior.

Forced swim test (FST)

FST was conducted in vertical glass cylinders (25 cm height, 10 cm diameter) filled with water to a 10 cm depth. Rats were placed individually in the cylinder for 6 min, and their immobility duration (lack of active escape attempts) was measured. Increased immobility time was interpreted as a marker of depressive-like behavior.

Statistical analysis

Data were analyzed using GraphPad Prism 9.0. Repeated-measures ANOVA was used for MWM acquisition trials. Two-way ANOVA followed by Bonferroni post hoc tests was applied for biochemical and behavioral comparisons. Pearson correlation analyses assessed relationships between behavioral and biochemical variables. Prior to conducting ANOVA analyses, data distributions were tested for normality using the Shapiro–Wilk test, confirming that all datasets met the assumptions of parametric testing. Results are presented as mean ± standard error of the mean (SEM), and significance was defined as $p < 0.05$.

Results

Behavioral measurements

The results of MWM showed that the mean latency to find the platform decreased gradually for all rats. Although this trend was not statistically significant, the reduction across four training days was more apparent in the HPOO and LPOO groups compared to control ($p > 0.05$) (Fig. 1A.1). In the probe trial, the time spent in the target quadrant was significantly increased in the HPOO group compared to control ($p < 0.01$), while the LPOO group did not differ significantly from control. The time spent in the opposite quadrant was significantly decreased in the HPOO group compared to control ($p < 0.05$), but LPOO again did not differ significantly from control. Although no significant difference was detected between HPOO and LPOO, the effects of HPOO were more pronounced than those of LPOO (Fig. 1A.2).

The results of OFT showed no significant difference between groups in time spent in the middle area ($p > 0.05$). However, both the HPOO and LPOO groups spent significantly less time in the periphery compared to control ($p < 0.01$ for both) (Fig. 2B.1). This suggests reduced anxiety-like behavior in both olive oil groups, with a more robust effect observed in HPOO.

The results of EPM demonstrated that the time spent in the open arms was significantly increased in the HPOO group compared to control ($p < 0.05$). The LPOO group showed a trend toward increased open arm exploration, but this did not reach statistical significance relative to control. Both HPOO and LPOO groups spent significantly less time in the closed

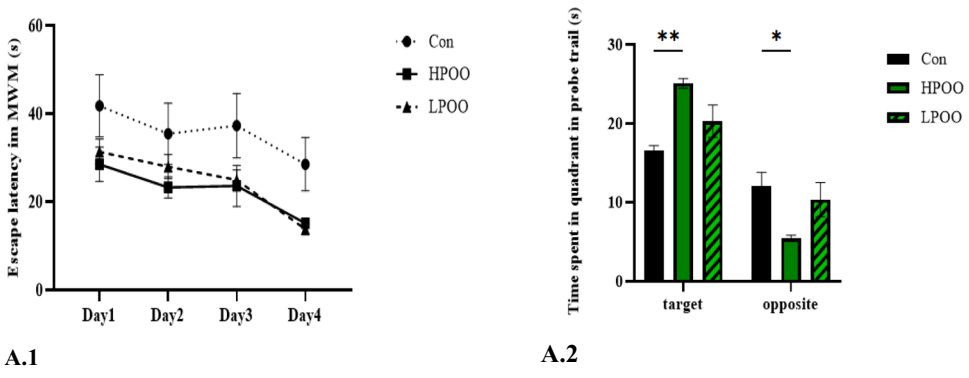

Fig. 1 The effects of HPOO and LPOO on learning and memory task. **A.1** Latency period to find the platform during four consecutive training days. **A.2** Time spent in target and opposite quadrants in the probe trial. (Significant differences are presented by $^{*}p < 0.05$ and $^{**}p < 0.01$). ($n = 6$–8 for each group)

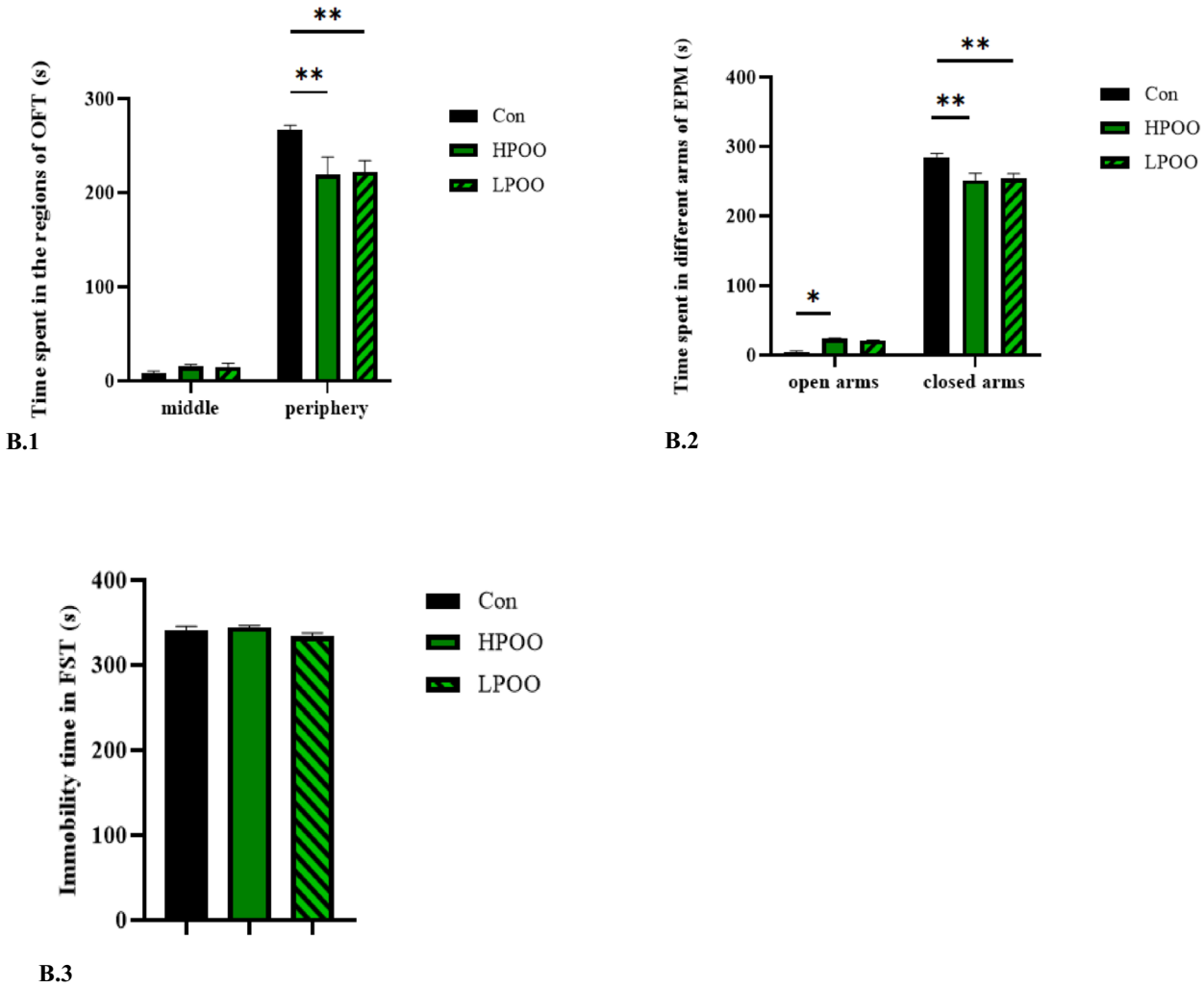

Fig. 2 Behavioral test results. Open field test (OFT) results. **B.1** The effects of HPOO and LPOO on time spent in the middle and the periphery walls of OFT. Elevated plus maze (EPM) results. **B.2** The effects of HPOO and LPOO on time spent in the open and closed arms of EPM. Forced swim test results (FST). **B.3** The effects of HPOO and LPOO on immobility time in FST. (Significant differences are presented by $*p<0.05$ and $**p<0.01$). ($n=6$–8 for each group)

arms compared with control ($p<0.01$ for both) (Fig. 2B.2). Although there was no significant difference between HPOO and LPOO, the anxiolytic effects were more pronounced in HPOO.
There was no significant difference between any groups in terms of immobility duration in FST (Fig. 2B.3).

Biochemical measurements of blood plasma and brain regions

TG levels were significantly decreased in both the HPOO and LPOO groups compared with control ($p<0.001$ for both). There was no significant difference in TG levels between HPOO and LPOO groups. Total cholesterol (TC) levels did not differ significantly across groups. The LDL/HDL ratio was significantly reduced in the HPOO group compared with control ($p<0.05$), whereas the LPOO group did not differ significantly from control. No significant differences were observed in ALT or AST levels between groups (Table 1).
Neuroinflammation in the hippocampus (HC) and prefrontal cortex (PFC) was evaluated via TNF-α/IL-10 ratio. In the HC, this ratio was significantly decreased in the HPOO group compared to both LPOO and control groups ($p<0.01$ vs control and $p<0.05$ vs LPOO). In the PFC, both HPOO and LPOO groups showed a significant reduction compared to control ($p<0.05$ for both). However, the magnitude of reduction was stronger in HPOO (Fig. 3).

Table 1 The effects of HPOO and LPOO on blood lipid profile

Parameters	TG (mg/dL)	TC (mg/dL)	LDL/HDL ratio	ALT (U/L)	AST (U/L)
HPOO	**43.09 ± 4.34****	79.83 ± 3.70	**0.26 ± 0.03***	38.19 ± 5.18	136.48 ± 11.65
LPOO	**44.52 ± 4.14****	83.60 ± 5.12	0.31 ± 0.06	37.73 ± 4.62	111.79 ± 3.41
Control	86.31 ± 3.72	66.00 ± 6.53	0.34 ± 0.01	51.10 ± 1.50	111.88 ± 4.59

Values are shown as means ± SEM. Significant differences are shown by *$p < 0.05$ and **$p < 0.01$ vs control group; $p < 0.05$ and $p < 0.01$ vs LPOO group

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *ALT* alanine transaminase, *TC* total cholesterol, *TG* triglyceride

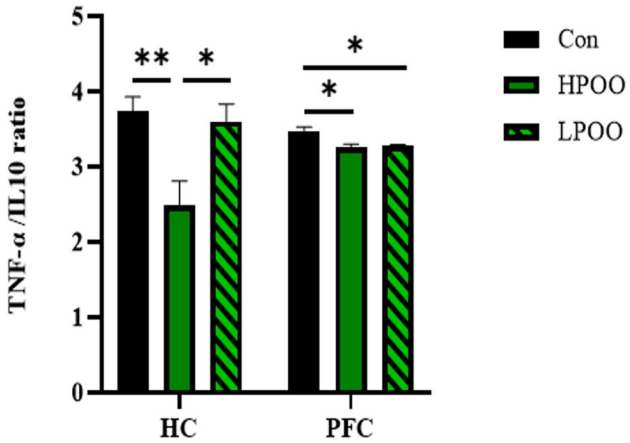

Fig. 3 Biochemical measurements. The effects of HPOO and LPOO on TNF-α/IL-10 ratio in hippocampus (HC) and prefrontal cortex (PFC). (Significant differences are presented by *$p < 0.05$ and **$p < 0.01$). ($n = 6$–8 for each group)

Correlation analysis

A strong negative correlation was found between TG levels and the time spent in open arms of the EPM ($r = -0.84$, $p < 0.01$) (Fig. 4A). Moderate positive correlations were observed between TG levels and both the time spent in closed arms of the EPM ($r = 0.48$, $p < 0.05$) and the time spent in the periphery walls of the OFT ($r = 0.58$, $p < 0.01$) (Fig. 4B, C). A moderate negative correlation was found between TG levels and the time spent in the target quadrant of the MWM probe trial ($r = -0.60$, $p < 0.01$) (Fig. 4D). Additionally, TG levels were positively correlated with the TNF-α/IL-10 ratio in PFC ($r = 0.64$, $p < 0.01$) (Fig. 4E).

The TNF-α/IL-10 ratio in both HC and PFC was moderately negatively correlated with time spent in the open arms of the EPM ($r = -0.73$, $p < 0.01$ for HC; $r = -0.57$, $p < 0.01$ for PFC) (Fig. 4F, G). In contrast, the TNF-α/IL-10 ratio in HC was positively correlated with the time spent in the periphery walls of the OFT ($r = 0.60$, $p < 0.01$) (Fig. 4H). The TNF-α/IL-10 ratio in HC showed a weak positive correlation with TG levels, though this was not statistically significant ($r = 0.434$, $p > 0.05$) (Fig. 4I).

Finally, although body weight was recorded weekly during the study, detailed data were not retained for statistical comparison across groups. Visual inspection during the experimental period did not reveal overt differences in growth trajectories between groups, and all animals showed expected age-related weight gain.

Discussion

This study is, to our knowledge, the first to compare the effects of HPOO and LPOO in male rats during the adolescent-to-young adult transition, a developmental stage that is highly plastic and vulnerable to inflammatory insults. While prior studies in aged or disease-model animals have demonstrated cognitive and anti-inflammatory benefits of polyphenol-rich olive oil, the current findings extend this knowledge to an earlier life stage and directly contrast the impact of high versus low polyphenol content. Our findings revealed that both HPOO and LPOO administration

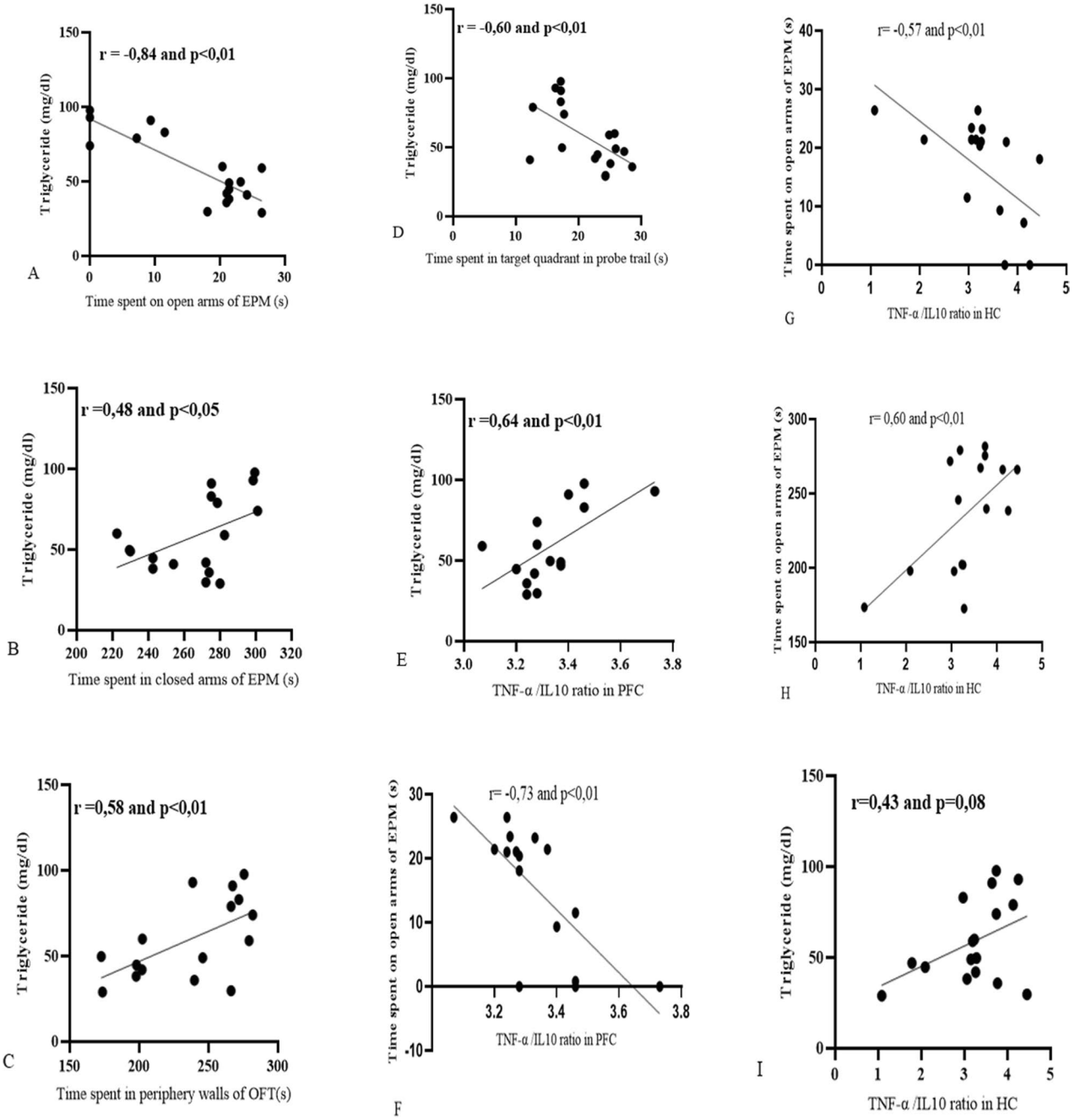

Fig. 4 Correlation analysis results. Correlation scatterplot between TG levels and time spent in the open arms of EPM (**A**). Correlation scatterplot between TG levels and time spent in the closed arms of EPM (**B**). Correlation scatterplot between TG levels and time spent in the periphery walls of OFT (**C**). Correlation scatterplot between TG levels and time spent in the target quadrant in probe trial (**D**). Correlation scatterplot between TG levels and TNF-α/IL-10 ratio in PFC (**E**). Correlation scatterplot between the time spent on open arms of EPM and TNF-α/IL-10 ratio in PFC (**F**). Correlation scatterplot between the time spent on open arms of EPM and TNF-α/IL-10 ratio in HC (**G**). Correlation scatterplot between the time spent on open arms of EPM and TNF-α/IL-10 ratio in HC (**H**). Correlation scatterplot between TG levels and TNF-α/IL-10 ratio in HC (**I**)

significantly reduced anxiety-like behaviors in open field and elevated plus maze tests, without significantly altering depression-like behaviors. These anxiolytic effects coincided with reductions in plasma triglyceride (TG) levels and TNF-α/IL-10 ratios in the prefrontal cortex (PFC) and hippocampus (HC), supporting the hypothesis that peripheral metabolic and central inflammatory parameters are intertwined in shaping emotional behaviors during adolescence.

Consistent with prior literature, the consumption of HPOO led to more pronounced improvements in spatial learning and memory, as evidenced by performance in the Morris water maze (MWM) test. This was accompanied by greater reductions in the TNF-α/IL-10 ratio within the hippocampus, a brain region intimately involved in learning and memory consolidation. TNF-α has been shown to impair long-term potentiation and synaptic function in the HC [28], while IL-10 is known to exert neuroprotective and anti-inflammatory effects [12]. The observed decrease in the TNF-α/IL-10 ratio suggests a shift toward an anti-inflammatory milieu, potentially facilitating neuroplastic processes underlying memory enhancement. These results are in alignment with previous work by Luceri et al. [13] and Cheema et al. [5], who demonstrated that EVOO rich in polyphenols enhanced memory and learning in rodent models.

Interestingly, although LPOO also decreased TG levels and anxiety-related behaviors, its effects on spatial memory and hippocampal inflammation were notably weaker. This finding suggests that while both olive oils may modulate systemic lipid metabolism, the cognitive benefits observed with HPOO are likely attributable to its higher polyphenol content. Polyphenols such as hydroxytyrosol and oleuropein have been shown to cross the blood–brain barrier and directly modulate neuronal signaling, oxidative stress, and inflammatory cascades [18, 21]. The absence of significant effects on depression-like behaviors in the forced swim test aligns with previous studies indicating that short-term dietary interventions may be insufficient to alter serotonergic and dopaminergic systems involved in affective processing [17].

The correlation analyses further supported a functional link between peripheral lipid regulation and central behavioral outcomes. Elevated TG levels were associated with increased anxiety-like behaviors and poorer performance in spatial memory tasks. This is in line with emerging evidence implicating dyslipidemia in neuropsychiatric disorders through mechanisms involving systemic inflammation, endothelial dysfunction, and hypothalamic–pituitary–adrenal (HPA) axis dysregulation [15, 24]. Additionally, the TNF-α/IL-10 ratio was positively correlated with anxiety measures, reinforcing its value as a neuroinflammatory index relevant to emotional regulation.

From a translational perspective, these findings underscore the potential of dietary polyphenols as modulators of adolescent neurodevelopment. While current research on the Mediterranean diet and olive oil has largely focused on adult or aging populations, our results suggest that early-life nutritional strategies may also yield benefits for mental health and cognitive outcomes. Importantly, these effects appear to be dose- and composition-dependent, highlighting the need for future studies to delineate the active components and optimal intake levels of olive oil for neuroprotection.

The study is not without limitations. Only two cytokines (TNF-α and IL-10) were assessed, providing a narrow view of the neuroimmune landscape. Including additional inflammatory mediators (e.g., IL-1β, IL-6, TGF-β) could yield a more comprehensive understanding of the underlying mechanisms. Moreover, while adolescent rats were used to reflect developmental vulnerability, future studies should explore sex differences and stress-induced models to simulate real-world conditions more closely. Additionally, mechanistic studies examining microglial activity, oxidative stress pathways, or transcriptomic changes in response to polyphenol-rich diets would help clarify the biological pathways involved.

Another important limitation concerns the translational relevance of the administered olive oil dose. Rats received 1 mL/day (~ 250 g total over the study), which, when scaled to body surface area, would correspond to approximately 0.16 L/day for a 40-kg adolescent human—far above typical dietary consumption levels [19]. Therefore, while the observed metabolic and neurobehavioral effects provide mechanistic insights, caution should be taken when extrapolating these findings directly to human nutrition. An additional limitation is the absence of detailed body weight data for statistical reporting, although no apparent differences in growth were observed across groups during the 8-week intervention. Finally, the study included only male rats. Sex-specific differences in lipid metabolism, inflammatory responses, and behavioral outcomes are well documented; therefore, the results cannot be directly generalized to female populations. Future studies should include both sexes to better evaluate the potential sex-dependent effects of olive oil polyphenols on neurodevelopment and behavior.

In summary, our findings demonstrate that HPOO, more so than LPOO, confers neuroprotective benefits during adolescence by improving spatial memory, reducing anxiety-like behavior, and modulating both lipid and inflammatory profiles. These results support the use of HPOO as a promising dietary strategy for enhancing cognitive and emotional health during a critical window of neurodevelopment.

Conclusion

In conclusion, this study demonstrated that both high- and low-polyphenol olive oil administration reduced anxiety-like behaviors but did not affect depression-like behaviors in adolescent rats. These anxiolytic effects were associated with reduced plasma triglyceride (TG) levels and decreased TNF-α/IL-10 ratios in both the prefrontal cortex (PFC) and hippocampus (HC). Notably, HPOO exerted more pronounced effects on spatial memory compared to LPOO, which appeared to be mediated by reductions in both serum TG concentrations and TNF-α/IL-10 ratio in the hippocampus. These findings suggest that improvements in lipid metabolism and central anti-inflammatory status may contribute to enhanced cognitive and emotional regulation during adolescence. Further studies involving broader panels of inflammatory cytokines and stress-induced adolescent models are warranted to elucidate the precise neuroimmune mechanisms underlying these effects. Moreover, olive oil polyphenols may hold promise as dietary modulators for mitigating anxiety and cognitive impairments during critical neurodevelopmental periods.

Author contribution N.U., M.A., and R.I. conceived the original idea and designed and supervised the experiments, analysis, and writing. R.I., B.K., G.G., and A.K. designed and performed the experiment and supervised the writing. S. Kizildag, B.K., S. Kandis, A.K., G.G., and R.I. performed behavioral tests and biochemical analysis. F.H., M.A., N.U., R.I., B.K., G.G., S. Kandis, and A.K. worked at animal sacrifice. R.I. and G.G. collected the data. R.I. and N.U. performed the statistical analysis. R.I. and M.A. wrote the manuscript; all authors read and approved the final draft submitted.

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Data availability Data will be made available on request.

Declarations

Ethics approval The animal procedures were approved by Dokuz Eylul University of School of Medicine Animal Care Committee (20/2017).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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