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Research Article

Novel Therapeutic Efficacy of Curcumin and Fish Oil (Omega-3 Fatty Acids) on Inhibiting Neuroinflammation and Neuronal Apoptosis via LPS Induction in BV-2 Cells

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Abstract

Neurological disorders, such as Parkinson's Disease and Alzheimer's Disease, have become more common in recent years, with the number of deaths due to neurological disorders increasing by about 39% since 1990 (GBD 2016 Neurology collaborators, 2019). Neuroinflammation (NI), an inflammatory response within the brain or spinal cord has been shown to accelerate the development of Parkinson's and Alzheimer's disease. Conventional pharmaceutical drugs to treat NI can be expensive and promote financial burdens for chronic treatment. The goal of this study was to identify possible natural product alternatives that control NI and stable cell morphology. One viable alternative is curcumin, a chemical compound found in turmeric (Curcuma longa). Another alternative is fish oil, a natural product enriched with omega-3 fatty acids. Pretreatment with curcumin or fish oil significantly attenuated lipopolysaccharide (LPS)-induced cytokine release from acute and chronically treated BV-2 microglial cells and reduced Caspase 3/7 expression in HT-22 neuronal cells.

Keywords: Curcumin, Fish oil, Neuroinflammation, Apoptosis, Microglia

Introduction

Neurological disorders, such as Parkinson's Disease and Alzheimer's Disease, have become more common in recent years, with the number of deaths due to neurological disorders increasing by about 39% since 1990 [1]. Additionally, another common cause of death is traumatic brain injury. Traumatic brain injury is among the most common causes of death in children and young adults (BIRI). Traumatic brain injuries can vary from something as familiar as a concussion to severe as a skull fracture or hemorrhage. The critical consequence as a result of both these things is neuroinflammation (NI). NI is an inflammatory response within the brain or spinal cord mediated by cytokines and other secondary messengers [2,3].

The physiological purpose of NI is to protect the brain and spinal cord from infections and eliminate cell debris caused by mishaps or diseases. Although NI is a response from the immune system to help protect the brain, long-term and uncontrolled NI can result in permanent brain damage and loss of brain function. For example, excessive NI has been shown to accelerate the development of diseases such as Parkinson's, Alzheimer's, and others. Another critical factor is cell morphology. Inflated neurons can have altered shape and suffer neural death due to NI, which can significantly affect their function. NI happens on a microscale due primarily to microglia releasing inflammatory cytokines. Although

several allopathic medicines help control NI, these drugs have several side effects. Specifically, a drug used to limit NI is Celecoxib, a commercially available COX-2 inhibitor, and its side effects include rashes, swelling of the face, heartburn, and fever. These conventional drugs can be decently expensive and leave financial burdens if treatment continues for months, which is common. There is a lack of research investigating the potential protective role of natural products on brain cells. Additionally, most of the allopathic treatments for NI are synthetic molecules, leading to a gap in research for the use of natural products on brain cells. Therefore, the aim of this work was to recognize and access possible natural product alternatives that control NI and stable neuronal apoptosis levels.

One viable alternative is curcumin, a chemical compound produced by turmeric (Curcuma longa). Prior research has found that curcumin, although not explicitly tested in the context of microglia and NI, has demonstrated anti-inflammatory effects in peripheral inflammatory diseases such as inflammatory bowel disease and arthritis [4]. Another alternative which will be compared to curcumin is fish oil. Omega-3 fatty acids are contained in fish oil and are through to promote its therapeutic effects. Prior research on animals and clinical interventions has found that, although not explicitly tested in microglia and NI, omega-3 fatty acids have strong anti-inflammatory properties [5]. Although these natural products show promising anti-inflammatory properties, neither has been used on brain cells to test for NI. It is hypothesized that both omega-3 fatty acids and curcumin will exert therapeutic effects by reducing NI and reducing neuronal apoptosis.

Methods

BV-2 immortalized mouse microglial cells and HT-22 immortalized mouse neuronal cells were plated overnight in a 24 well plate at 100,000 and 12,500 cells per well respectively. BV-2 cells were incubated with either a negative control (cell media), a positive control of 100nM of lipopolysaccharide (LPS), 100nM of LPS and descending concentrations of curcumin (50, 25, and 12.5μM) or 100nM of LPS and descending concentrations of fish oil (0.05%, 0.01%, and 0.002% v/v) run in triplicate based on previous studies [6,7]. Conditioned media was collected from BV-2 cells 3 and 20 hours after the incubation of the above treatment conditions and were assessed for tumor necrosis factor-alpha (TNF-α) and interleukin-6 cytokine levels using enzyme-linked immunoassay (ELISA). Based on results from the treatment concentration response curve, maximally effective concentrations of curcumin and fish oil were incubated together with LPS to determine potential synergistic effects on cytokine release. 20-hr post incubation media from either LPS alone, control or maximally effective concentrations of curcumin or fish oil were applied to HT-22 cells. Caspase 3/7 expression in HT-22 were measured 24 hours after conditioned media incubation using Caspase 3/7 green dye to assess apoptosis [8-10].

Results

Conditioned media from LPS treated BV-2 cells showed time-dependent increases in both TNF-α (Figure 1a and 1b) and IL-6 (Figure 2a and 2b) levels. Following acute exposure to LPS (3 hrs), conditioned media from BV-2 displayed a significant increase in TNF-α (2,010.70pg/m) compared to control conditions (8.58pg/mL, Figure 1a). Treatment with curcumin or fish oil reduced TNF-α in a concentration dependent manner (Figure 1a), with the strongest inhibitory effect observed at the maximal concentrations tested (16.72pg/mL for the 50μM curcumin/100nM LPS condition and 1,660.99pg/mL for the 0.05% fish oil/100nM LPS condition). Acute exposure to LPS (3hr) did not increase IL-6 levels (14.29pg/mL, Figure 2a) compared to control (12.25pg/mL) and there was no significant effect of curcumin or fish oil on IL-6 levels at any concentration at this time point [11-15].

After 20 hours after LPS exposure, conditioned media from BV-2 cells displayed a significant increase in both TNF-α (5,254.97pg/mL, Figure 1b) and IL-6 levels (1,073.54pg/mL, Figure 2b) compared to control conditions (137.19pg/mL and 13.94pg/mL respectively). Even after 20 hours, treatment with curcumin or fish oil significantly reduced TNF-α levels in a concentration dependent manner (Figure 1b), with maximal reductions observed at the highest concentrations of curcumin (2,591.74pg/mL for the 50μM curcumin/100nM LPS condition) or fish oil (1,258.74pg/mL for the 0.05% fish oil/100nM LPS condition). Additionally, treatment with curcumin or fish oil significantly reduced IL-6 in a concentration dependent manner (Figure 2b), with maximal reductions observed at the highest concentrations (14.81pg/mL for the 50μM curcumin/100nM LPS condition and 73.53pg/mL for the 0.05% fish oil/100nM LPS condition) [16].

Based on these results, maximal concentrations of curcumin (50μM) and fish oil (0.05%) were incubated together with LPS (100nM) to determine potential synergistic effects on TNF-α (Figure 3) and IL-6 levels (Figure 4) at both short- and long-term exposure [17]. It was determined that incubation with these maximally effective concentrations did not further lower TNF-α levels at either time point tested (Figure 3a and 3b), nor IL-6 levels at the long-term exposure condition (Figure 4b). HT-22 cells incubated with 20-hr LPS-conditioned media showed a significant increase in the expression of the apoptosis marker Caspase 3/7 (13.17% of cells), as indicated by increased green dye fluorescent expression compared to control (1.22% of cells) (Figure 5). Conditioned media from the 50μM curcumin/100nM LPS or 0.05% fish oil/100 nM LPS condition showed significant reductions in Caspase 3/7 expression (7.25% and 8.13% respectively) in comparison to LPS (13.17%) [18].

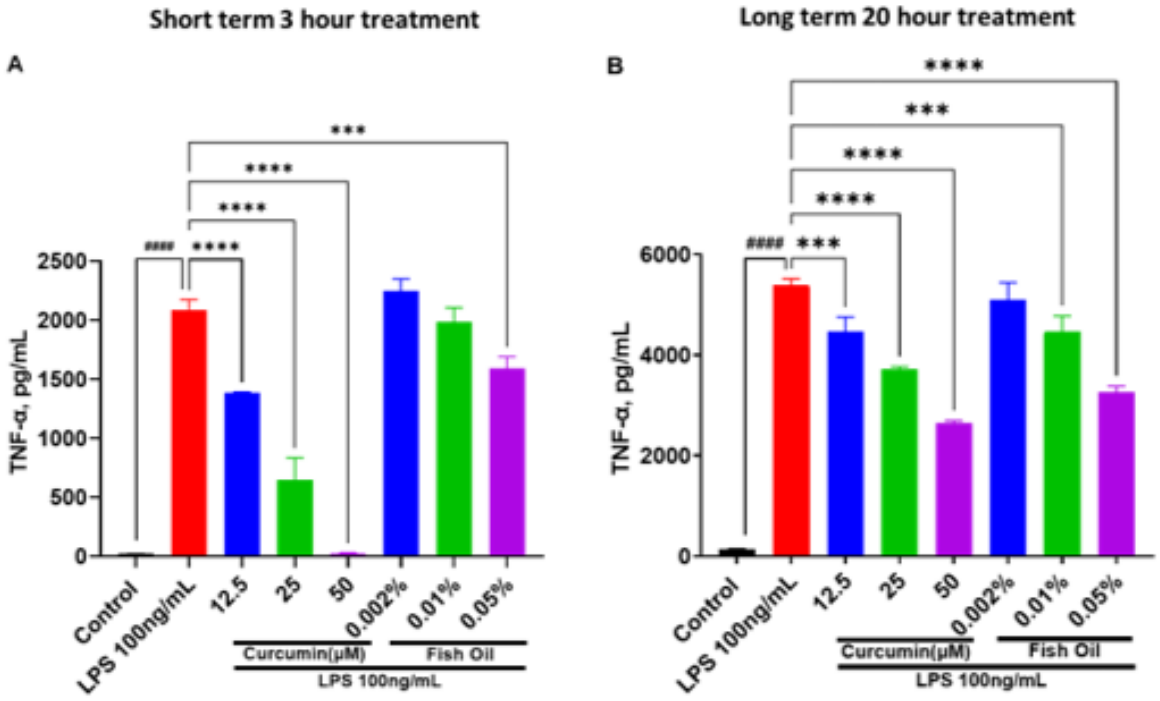

Figure 1: Increased TNF-α Levels in Response to LPS Stimulation Are Reduced with Curcumin or Fish Oil Treatment.
Note*: Short term (A) and long term (B) co-treatment with increasing concentrations of curcumin or fish oil significantly reduces TNF-α levels associated with LPS exposure in BV-2 cells. One-Way ANOVA; post-hoc comparison between LPS (100ng/mL) and control, ### = $p < 0.001$; post-hoc comparison between LPS (100ng/mL) and co-treatment conditions, *** = $p < 0.001$, **** = $p < .0001$

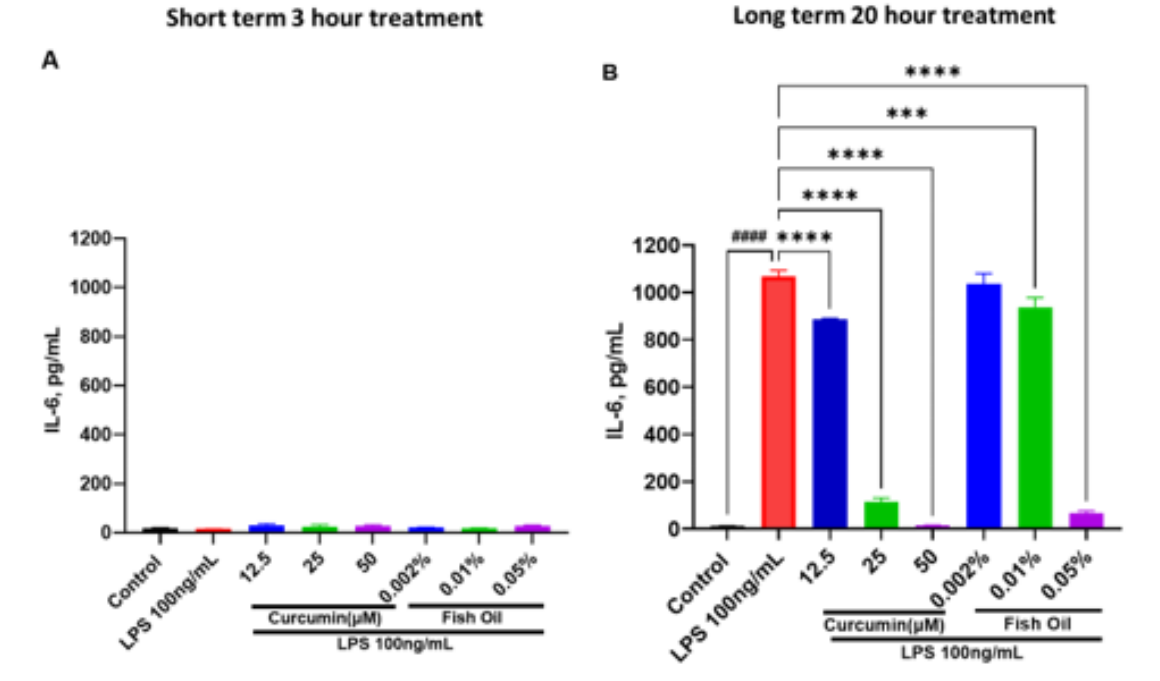

Figure 2: Increased IL-6 Levels in Response to LPS Stimulation Are Time-Dependent and Reduced with Curcumin or Fish Oil Treatment.
Note*: Long term (B) but not short term (A) exposure to LPS induces IL-6 secretion in BV-2 cells. Co-treatment with increasing concentrations of curcumin or fish oil significantly reduces IL-6 levels associated with LPS exposure at long term exposure (B). One-Way ANOVA; post-hoc comparison between LPS (100ng/mL) and control, ### = $p < 0.001$; post-hoc comparison between LPS (100ng/mL) and co-treatment conditions, *** = $p < 0.001$, **** = $p < .0001$.

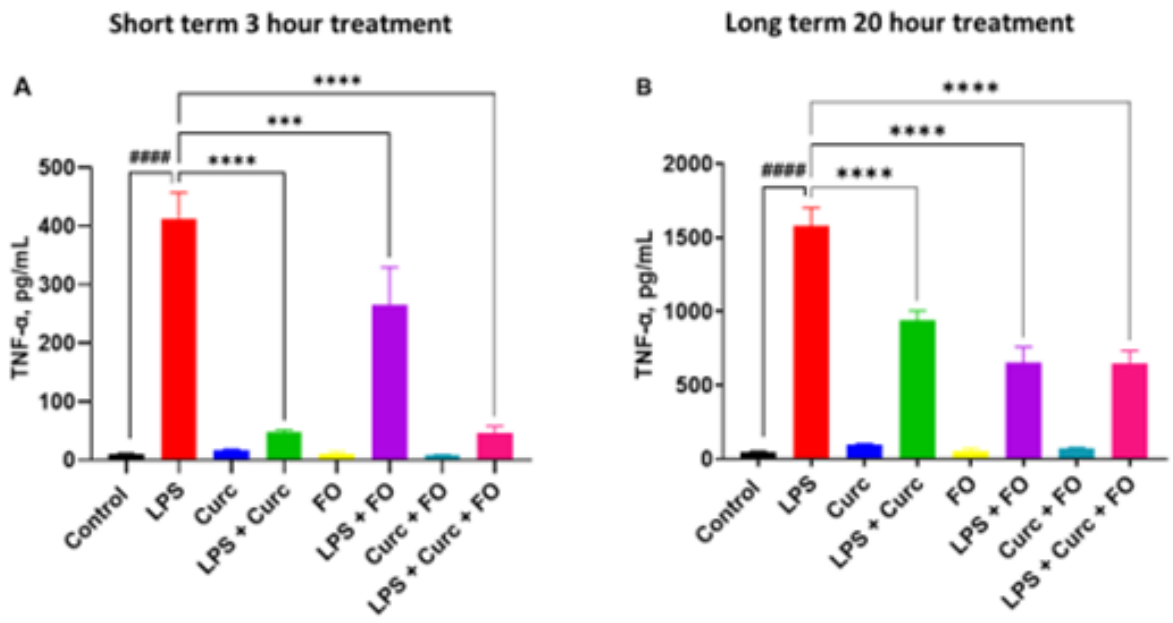

Figure 3: Combined Treatment with Curcumin and Fish Oil Does Not Produce Additional Reductions in TNF-α Levels.
Note*: Combined treatment with the maximal effective concentration of curcumin (50μM) and fish oil (0.05%) does further reduce TNF-α levels associated with short (A) or long term (B) exposure to LPS in BV-2 cells. One-Way ANOVA; post-hoc comparison between LPS (100ng/mL) and control, ### = p < 0.001; post-hoc comparison between LPS (100ng/mL) and co-treatment conditions, *** = p < 0.001, **** = p < .0001.

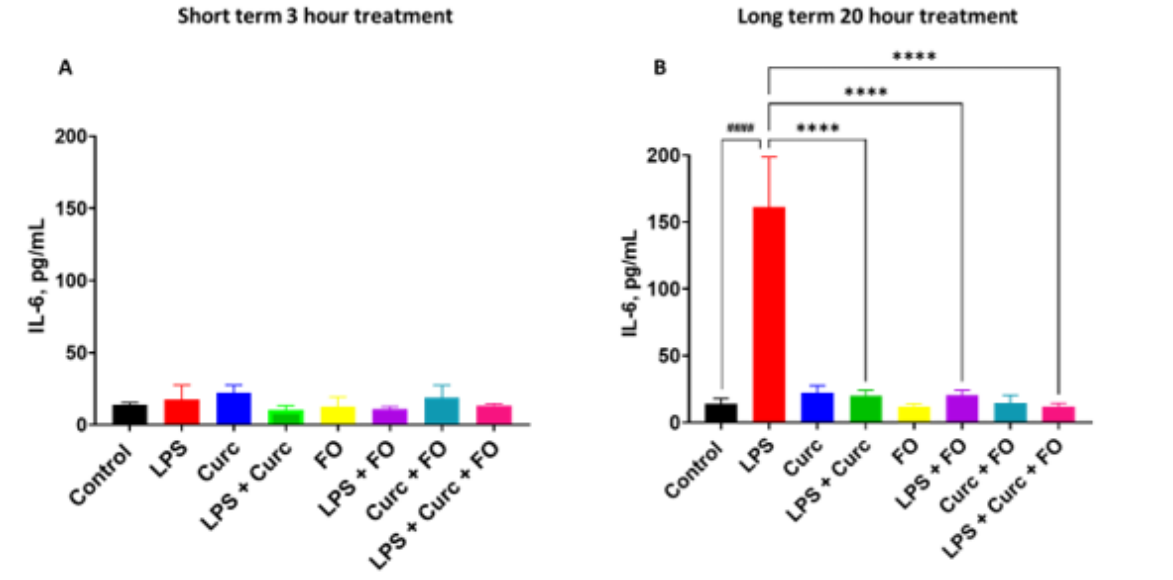

Figure 4: Combined Treatment with Curcumin and Fish Oil Does Not Produce Additional Reductions in IL-6 Levels.
Note*: Combined treatment with the maximal effective concentration of curcumin (50μM) and fish oil (0.05%) does further reduce IL-6 levels associated with long term (B) exposure to LPS in BV-2 cells. One-Way ANOVA; post-hoc comparison between LPS (100ng/mL) and control, ### = p < 0.001; post-hoc comparison between LPS (100ng/mL) and co-treatment conditions, *** = p < 0.001, **** = p < .0001.

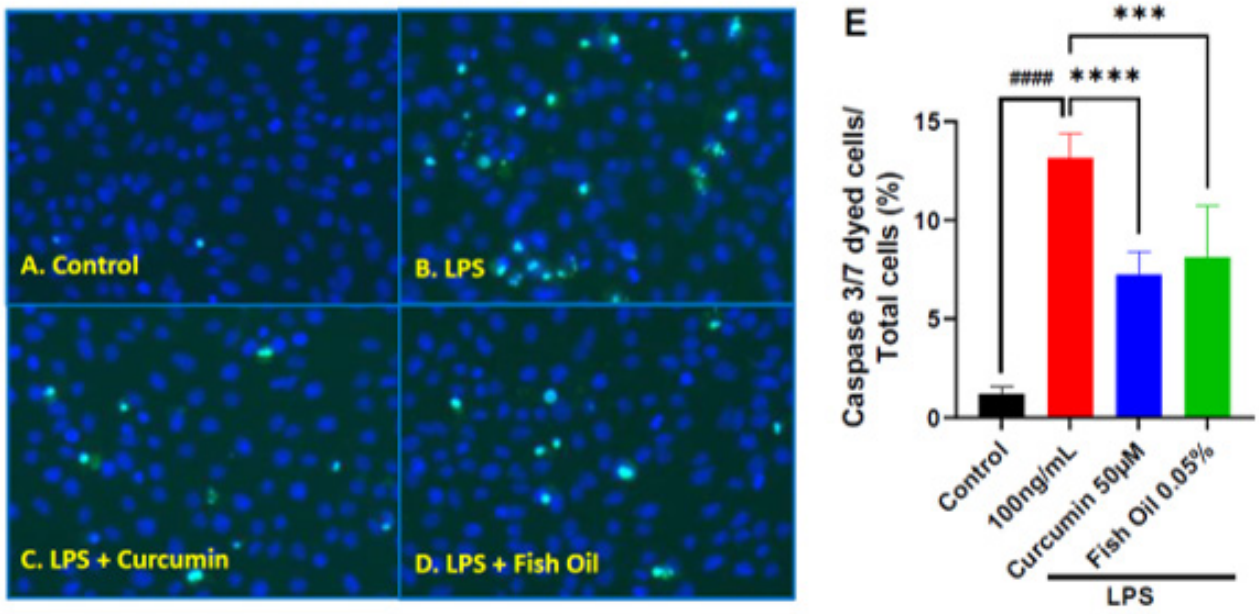

Figure 5: Treatment with Curcumin or Fish Oil Significantly Reduces Neuronal Apoptosis From LPS-Conditioned Media.
Note*: Representative image of Caspase 3/7 green dye fluorescent detection in HT-22 cells. LPS induces a significant increase in the apoptotic marker Caspase 3/7, which is significantly reduced with either curcumin or fish oil co-treatment. One-Way ANOVA; post-hoc comparison between LPS (100ng/mL) and control, ### = $p < 0.001$; post-hoc comparison between LPS (100ng/mL) and co-treatment conditions, *** = $p < 0.001$, **** = $p < .0001$.

Discussion

Curcumin and fish oil co-treatment significantly attenuated the LPS-associated TNF-α increase in a concentration-dependent manner. Notably, a significant LPS- associated increase in IL-6 levels after short 3 hours of treatment was not observed. However, long-term 20-hour LPS-treatment promoted high IL-6 levels that were suppressed by both curcumin and fish oil treatments in a concentration-dependent manner. Results demonstrate that the LPS-only control medium significantly increased neuronal apoptotic cells, but curcumin and fish oil co-treatments attenuated apoptosis. Intriguingly, although curcumin and fish oil were equally efficacious in lower IL-6 levels to near control, curcumin was more effective than fish oil to reduce acute TNF-α levels. When combined, there was no additional effect on lowering TNF-α or IL-6, importantly, it appears that fish oil did not interfere with curcumin's efficacy either, suggesting distinct mechanisms of action. LPS conditioned media induced significant apoptosis in HT-22 cells, both curcumin and fish oil significantly reduced (about 50%) but did not completely attenuate this effect. This is consistent with a significant reduction (about 50%) but not a complete attenuation in TNF-α levels at the 20 hr timepoint [19-21].

Certain limitations of the study should be noted. The maximally effective concentration of fish oil and curcumin was detected for reducing TNF-α for the 3hr but not 20hr timepoint, suggesting the efficacy of both products may be time sensitive or possibly surmountable [22]. Intriguingly, maximal efficacy for reducing IL-6 was determined at the 20hr timepoint. It is possible that curcumin and fish oil block the initiating mechanism for IL-6 release at the 3hr timepoint, thereby reducing release at the later 20hr timepoint. It has been demonstrated that TNF-α initiates IL-6 secretion in rat C6 glioma cells, therefore, the efficacy of the natural products in attenuating IL-6 secretion may be dependent on their ability to block TNF-α early on. Future studies should confirm this potential mechanism. Further, these studies were conducted in immortalized mouse cells without co-culturing, which may not completely mimic conditions observed in vivo. Future studies should be conducted in vivo, utilizing mouse models of inflammation such as LPS exposure, which should mimic the in vitro response, but may display interactive effects [23,24]. Finally, the mechanism of action for reducing TNF-α in particular with curcumin and fish oil was beyond the scope of the study, but future studies blocking oxidative stress or intracellular signaling may help understand potential efficacy differences between the two for cytokine release.

Conclusion

Curcumin and fish oil effectively reduce LPS-induced cytokine release in BV-2 cells, attenuating cytokine-induced neuronal apoptosis in HT-22 cells, suggesting their neuroprotective effects may be dependent on inhibiting microglial activation.

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