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(Original Research)

The Impact of Menhaden Fish Oil on Brain Tauopathy in Streptozotocin-Lipopolysaccharide-Induced Rodent Model of Alzheimer's Disease

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Abstract

Background: Tauopathies, including Alzheimer's disease (AD), are neurodegenerative conditions characterized by the accumulation of abnormally phosphorylated tau protein, often exacerbated by comorbid metabolic disorders such as diabetes mellitus. This study evaluated the neuroprotective effect of Menhaden Fish Oil (MFO), a rich source of preformed omega-3 fatty acids, on tau pathology in a streptozotocin-lipopolysaccharide (STZ-LPS)-induced rat model that mimics diabetes-associated AD. **Methods:** Thirty male Wistar rats were grouped into five distinct groups and subjected to treatment with either normal saline or MFO (1 g/kg or 3 g/kg) for 21 days, followed by biochemical, immunofluorescence microscopy, and RT-qPCR analyses. **Results:** MFO administration significantly reduced total tau protein levels and tau hyperphosphorylation at Ser202 in both the cortex and hippocampus. It also upregulated AKT-1 expression while downregulating GSK-3 and CASP-3, suggesting modulation of the AKT/GSK-3 signaling pathway. **Conclusions:** These findings demonstrate that MFO supplementation mitigates tau pathology and neuronal apoptosis in a metabolic-inflammatory model of AD, supporting its potential as a dietary neuroprotective strategy.

Keywords

Alzheimer's disease, Brain, Diabetes, Omega-3 fatty acid, Tau protein

1. Introduction

Tauopathies, particularly Alzheimer's disease (AD), are a major focus of neurodegenerative research due to their profound impact on cognition, memory, and quality of life. AD, the leading cause of dementia worldwide, is marked by abnormal protein aggregation and progressive neuronal loss [1]. Among the pathological hallmarks of AD, tau protein dysfunction plays a pivotal role. Under normal conditions, tau stabilizes neuronal microtubules and supports axonal transport [2], but in disease states, it becomes hyperphosphorylated and aggregates into neurofibrillary tangles (NFTs) that disrupt synaptic function and contribute to neuronal degeneration [3]. Tau-related pathology is also observed in other neurodegenerative disorders, including frontotemporal dementia and Parkinson's disease, indicating a broader relevance of tau dysfunction in brain health [4]. As AD prevalence continues to rise, understanding the mechanisms underlying tau pathology and developing interventions to counteract it remain crucial.

Mounting evidence suggests that metabolic dysfunction, particularly diabetes mellitus (DM), contributes significantly to AD development and progression. Insulin resistance and impaired insulin signaling in the brain have been shown to promote tau hyperphosphorylation, linking DM to tauopathy [5-6]. Experimental models have consistently demonstrated elevated tau phosphorylation in the cortex and hippocampus of diabetic animals [7-8]. Notably, various models exhibit distinct phosphorylation profiles; for example, Otsuka Long Evans Tokushima Fatty rats show increased Ser199/202 and Ser396 phosphorylation [9], while tau transgenic mice treated with a high-fat diet exhibit hyperphosphorylation at Ser396/404 [10], Streptozotocin (STZ)-induced diabetic mice exhibit hyperphosphorylation at multiple sites, including Thr181, Ser199, Ser202, and Ser396/404 [11]. These findings underscore the interplay between metabolic dysfunction and tau aggregation, reinforcing the view that AD represents both a neurodegenerative and metabolic disorder [12].

One of the main regulators of tau hyperphosphorylation is glycogen synthase kinase-3 (GSK-3), a serine/threonine kinase that phosphorylates tau at multiple sites. GSK-3 is hyperactive in AD brains and is implicated in both tau aggregation and amyloid-ß pathology [13-14]. Given its central role, modulation of the Ak strain transforming (AKT)/GSK-3 pathway has been widely investigated as a therapeutic strategy for AD. Activation of Akt leads to the inhibitory phosphorylation of GSK-3, reducing tau phosphorylation and neuronal damage [15]. However, direct pharmacological inhibition of GSK-3 has been challenging due to toxicity and lack of specificity. This has prompted exploration of natural compounds, particularly omega-3-rich dietary interventions, as safer alternatives to modulate tauopathy.

Fish oil (FO), rich in long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has demonstrated neuroprotective effects through anti-inflammatory, antioxidant, and anti-apoptotic mechanisms [16-17]. Animal studies have shown that FO supplementation can reduce tau hyperphosphorylation and preserve neuronal function. In 5xFAD transgenic mice, short-term FO supplementation decreased hyperphosphorylated tau in the parietal cortex by 36% compared to untreated controls [18]. Similarly, FO combined with curcumin attenuated tau phosphorylation and improved insulin signaling by inhibiting c-Jun N-terminal kinase (JNK) pathways [19]. Human studies also indicate that DHA-rich FO can reduce GSK-3 activity and improve insulin sensitivity in overweight adults, suggesting potential cross-talk between metabolic and neurodegenerative pathways [20]. These findings highlight the potential of FO as a dietary intervention for AD prevention and management.

Menhaden fish oil (MFO) is regarded as a sustainable and environmentally friendly marine resource that provides omega-3 fatty acids (EPA and DHA) and is known for its health benefits, including cardiovascular protection and anti-inflammatory effects [21]. In contrast to plant-based sources like flaxseed or chia, which only provide alpha-linolenic acid (ALA) that must be converted into EPA and DHA through inefficient enzymatic processes in humans [22]. MFO, like other forms of FO that contain pre-formed DHA and EPA, can directly increase active fatty acids within cells and tissues effectively [23]. In comparison to other marine oils, such as cod oil or tuna oil, MFO provides a superior concentration of EPA and DHA, often containing between 1200mg and 2000mg of EPA and DHA per 100g, allowing for lower therapeutic dosages [24]. This unique composition strengthens its anti-inflammatory potential and its ability to modulate key signaling pathways such as AKT/GSK-3 and caspase-3 (CASP-3), which are critical for mitigating tau hyperphosphorylation and neuronal apoptosis. These biochemical advantages make MFO a promising candidate for research on complex neurodegenerative processes, particularly those associated with DM-related AD.

Although FO has been extensively studied, the molecular mechanisms by which MFO modulates tau pathology in models combining metabolic dysfunction and neuroinflammation remain underexplored. Previous studies have often focused on amyloid pathology rather than tauopathy and rarely examined dose-dependent effects or early phosphorylation sites such as Ser202, which are strongly associated with tau misfolding [25-26]. Furthermore, the direct influence of MFO on the expression of genes related to the AKT/GSK-3/CASP-3 pathway is still unclear. To address these gaps, the present study investigates the neuroprotective effects of MFO in

an STZ-LPS rat model that integrates metabolic dysfunction (STZ-induced diabetes) and neuroinflammation (LPS exposure), thus better replicating the multifactorial nature of DM-associated AD [27]. It is hypothesized that MFO supplementation will reduce total tau levels, decrease Ser202 phosphorylation, and regulate the expression of AKT-1, GSK-3, and CASP-3. Through ELISA, immunofluorescence, and RT-qPCR analyses, this study aims to provide novel insights into how MFO, with its unique omega-3 composition, can act as a multi-target neuroprotective agent.

2.1 General materials

Menhaden fish oil (Cat no: F8020) and lipopolysaccharide (LPS) (Cat no: L2630) were purchased from Sigma-Aldrich company (USA). Streptozotocin (Cat no: SC-200719) was purchased from Santa Cruz Biotechnology Company (USA). The Pro-prep protein extraction solution (Cat no: 17081) was purchased from Intron Biotechnology Company (South Korea). The total tau protein (Cat no: E1191Ra) was provided by Bioassay Technology Laboratory Company (China). The primary antibody against phospho-tau (Ser 202, Thr205) (Cat no:MN1020) from Thermo Fisher Scientific Company (USA). Meanwhile, the secondary antibody against Goat Anti-Rabbit IgG H&L (TRITC) (Cat no: ab6718) was purchased from Ab Cam Company (UK). Meanwhile, for the real-time-qualitative polymerase chain reaction (RT-qPCR) method, the Total RNA mini kit (Cat no: RT100) was purchased from Geneaid Biotechnology Company (Taiwan), ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Cat no: FSQ-301) from Toyobo Company (Japan), and SensiFAST SYBR No-ROX Kit (Cat no: BIO-98005) from Bioline Company (UK). Other chemicals used throughout this study were analytical grade.

2.2 Experimental animals

Male Wistar rats weighing 250-280 g were purchased from Anilab, Indonesia. The animals were acclimated for seven days in the Laboratory for Experimental Animal Development of the Faculty of Medicine (Universitas Brawijaya, Indonesia) before the experiment. Under conditions of well-ventilated conditions with 12-hour light/dark cycles, rats were housed in cages, with three rats per cage. They were also given unrestricted access to water and standard rat chow (Rat bio, Citra Ina Feedmill, Indonesia) on an *ad libitum* basis. The animal bedding was made of sawdust (Chipsi, Germany, JRS Germany GmbH & Co.) and was changed every morning during the study. The sample size (n=6 per group) was selected based on the resource-equation method [28] to account for attrition and maintain adequate statistical power (DF = 25). The Institutional Animal Care and Use Committee (IACUC) at Universiti Putra Malaysia, Selangor, Malaysia, approved all experimental care and procedures for the animal investigations (Approval NO: UPM/IACUC/AUP-R017/2022).

2.3 Experimental design and procedures

A total of thirty rats were randomly selected (n=6 for each group) into five groups: 1) Animal received Normal saline (NS) induction+NS oral (Control group), 2) Animal received NS induction+MFO with dosage of 3 g/kg (MFO control group), 3) Animal received STZ-LPS induction+NS oral (STZ-LPS group), 4) Animal received STZ-LPS induction+MFO with dosage of 1 g/kg (MFO 1g/kg group), 5) Animal received STZ-LPS induction+MFO with dosage of 3 g/kg (MFO 3g/kg group). Animal welfare was closely monitored throughout the STZ-LPS induction period. Body weight, food and water intake, and clinical signs of morbidity (e.g., lethargy, abnormal grooming, posture) were assessed daily. Humane endpoints were applied, and any rat exhibiting >20% body weight loss or severe distress was euthanized according to institutional ethical guidelines.

At the end of the experiment, all rats were euthanised using ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Following euthanasia, a necropsy was conducted. The brain samples were carefully isolated, washed, and separated by sagittal sections into two parts: left and right hemispheres. The left hemispheres were submerged in a 10% neutral buffered formalin (NBF) fixative solution at room temperature for 24 hours for immunofluorescence histological analysis. Meanwhile, the right hemispheres were kept frozen at -80°C until the ELISA and RT-PCR assays were conducted. Figure 1 illustrates the experimental design.

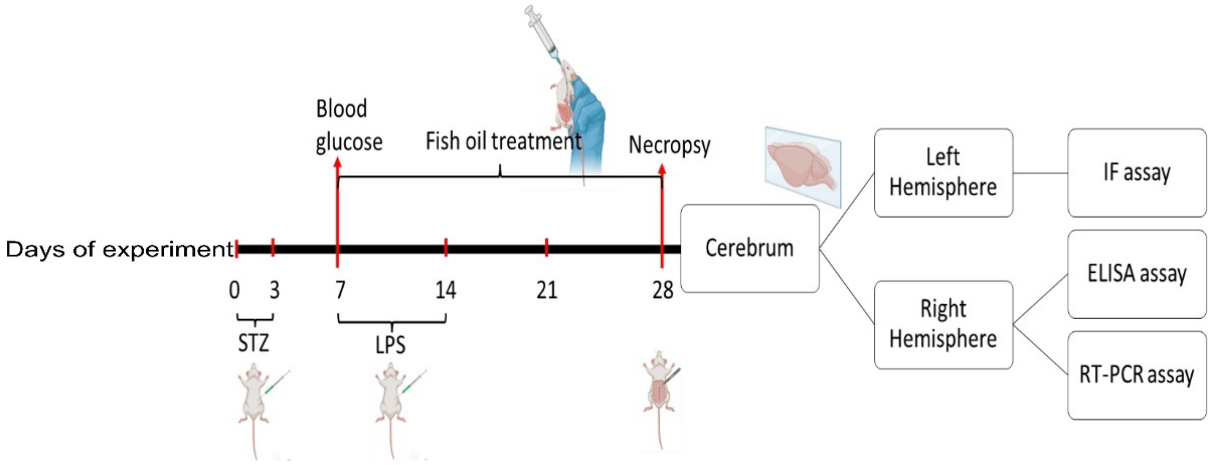


Figure 1. A simplified illustration of the experimental design timeframe. STZ was administered from days 1 to 3, followed by LPS injections from days 7 to 13. Fish oil treatment was initiated on day 7 and continued until day 27, with the experiment concluding on day 28. Abbreviations: STZ: streptozotocin; LPS: lipopolysaccharide; IF: immunofluorescence; ELISA: enzyme-linked immunosorbent test; RT-PCR: real-time polymerase chain reaction.

2.3.1 Induction of Diabetes Mellitus

Multiple intraperitoneal (i.p.) injections of freshly prepared STZ at a dose of 45 mg/kg were administered to overnight fasted rats for three consecutive days to induce diabetes mellitus [29]. STZ was dissolved in 0.1 mol/L sodium citrate buffer solution (pH 4.5). Following one week of STZ administration, blood was obtained from the tail vein to assess fasting blood glucose levels in rats that had been fasted for eight hours, utilizing a digital blood glucose meter (GlucoDr, Korea). Only rats with fasting blood glucose levels of 250 mg/dL or above were categorized as diabetic and included in the experiment.

2.3.2 Induction of Alzheimer's Disease

The onset of Alzheimer's disease (AD) was induced by daily administration of 250 µg/kg of LPS over a period of seven consecutive days [30]. LPS was administered intraperitoneally after being diluted in a physiological saline solution (0.9% NaCl). Throughout the administration of LPS, the body temperature of the animal was carefully monitored each morning. The success of AD induction was confirmed through behavioral assessment using the Y-maze test conducted after LPS injection. STZ-LPS-induced rats showed a significantly higher number of entries into the familiar arm compared to the start and novel arms, with the longest duration spent in the start arm and the shortest in the novel arm, indicating spatial memory impairment characteristic of AD-like cognitive deficits.

2.4 Brain protein extraction preparation

The extraction of brain proteins was conducted utilizing a Pro-prep protein extraction solution, involving the collection of 10-20 mg of cortical and hippocampus tissues. The tissues underwent homogenization in 600 µL of Pro-prep solution, subsequently transferred to a microtube and centrifuged at 13,000 rpm for 10 minutes. The samples underwent incubation for a duration of 30 minutes, followed by centrifugation at 13,000 rpm for a period of five minutes. The supernatant was subsequently transferred to a new 1.5 mL microtube and prepared for ELISA analysis. The procedure adhered to the specifications provided by the manufacturer.

2.5 Total tau protein analysis

All reagents, standard solutions, and brain samples were meticulously prepared in accordance with the kit instructions and allowed to acclimate to room temperature naturally. Initially, 50 µL of the standard solution was added to the designated well for standards. Subsequently, 40 µL of the brain sample was added to the sample wells, followed by the addition of 10 µL of rat tau protein antibody. Fifty microliters of Streptavidin-HRP

were added to the standard and sample wells and subsequently homogenized. The plate was carefully sealed and incubated for 60 minutes at a controlled temperature of 37℃. The plate underwent a rigorous cleaning process, being washed five times with a wash buffer. Each well was soaked with 300 μL for each wash for 30 seconds. Subsequently, the plate should be tapped onto paper towels before the sequential addition of 50 μL of substrate solution A and solution B to each well. Subsequently, the plate must be should be incubated with the new sealer in a dark environment for 10 minutes at a temperature of 37℃. Ultimately, it is essential to introduce 50 μL of stop solution into each well to halt the reaction before measurement. The absorbance at a wavelength of 450 nm ought to be assessed utilizing a Bio-Rad microplate reader (USA). The final cytokine concentrations were expressed as ng/g of wet tissue.

2.6 Phosphorylated tau protein (Ser 202) immunofluorescence analysis

The unstained brain tissues were heated for an hour at 60°C. The samples were then twice submerged for ten minutes each in xylol. The samples were then twice immersed in xylol for ten minutes each. After that, the samples must be rehydrated with ethyl alcohol in descending graded series, beginning at a concentration of 100% (2 changes), then 90%, 80%, and 70% every five minutes. The samples were rinsed three times using phosphate-buffered saline (PBS). The slides were subsequently covered with a solution of PBS Triton-X 100 at a concentration of 0.1%, which was allowed to remain for five minutes. The slides were subsequently covered with a 1% BSA solution and allowed to incubate for 30 minutes at room temperature. The slides are subsequently incubated with primary antibodies targeting tau phosphorylation at Ser202 (pSer202) at a dilution of 1:1000 for an extended overnight duration at 4°C. Following three washes with PBS, the slides underwent a 30-minute incubation at room temperature with secondary antibodies of Goat Anti-Rabbit IgG H&L at a dilution of 1:1000. For a duration of five minutes, DAPI was employed at a dilution of 1:1000 as a counterstain on the sections. Subsequently, the slides underwent a triadic washing process with PBS, mounted, and subsequently covered with glass. A fluorescence microscope (Olympus FV1000, Olympus, Tokyo, Japan) was employed to capture images of rat brain sections. In order to assess the immunoreactivity associated with tau phosphorylation at Ser202, the number of cells in each brain section was counted as the number of positive cells through the utilization of ImageJ software. Quantification of αß42 immunoreactivity was performed using ImageJ software. A blinded observer manually defined the region of interest (ROI) corresponding to the cortical area on each immunofluorescence image at 200× magnification (image resolution: 800 × 600 pixels). The red fluorescence channel, representing pSer202 signal, was analyzed using the 'Measure' function to obtain the integrated density values. These values were used to calculate the percentage of the ROI area exhibiting positive pSer202 staining. For each animal, five non-overlapping sections were analyzed, and the resulting percentages were averaged to yield a single representative value per subject.

2.7 Real time-qualitative polymerase chain reaction (RT-qPCR) procedures

The designated primer pairs for three genes associated with the tau phosphorylation pathway, along with one reference gene, were generated utilizing the Integrated DNA Technologies program (see Table 1). Gene sequences were derived from data sourced from the Gene Bank™ database pertaining to *Rattus norvegicus*. The gene specificity of the primer sequences was verified through BLAST searches, which revealed a lack of multi-locus matching at each individual primer site. The beta-actin gene (ACTB) served as an internal control or housekeeping gene to assess the quantity and quality of cDNA, which was then utilized as a standard for estimating the expression of candidate genes. Melt curve analysis was performed to ensure the specificity of amplification, with single, sharp peaks indicating the absence of primer-dimers or nonspecific products. Amplification efficiency for each primer pair was determined using standard curves, yielding efficiency values within the acceptable range of 90–110%. The ACTB gene was selected as the housekeeping gene due to its stable expression across all experimental groups, as confirmed by preliminary Ct analyses and supported by prior studies using STZ-LPS models.

Table 1. The parameters that were determined from the analysis of RT-qPCR data, along with the primers designated for the APP target genes and a reference gene.

Gene symbol	Gene name	Primer Forward (F)/ Reverse ®	Genbank	Amplicon size (bp)
GSK-3	Glycogen synthase kinase-3 beta	ACCTGCCCTCTTCAACTTTAC CACGGTCTCCAGCATTAGTATC	NM_032080.1	149
AKT-1	AKT serine/threonine kinase 1	GCTGGAGGACAACGACTATG CTTCTCATGGTCCTGGTTGTAG	NM_033230.3	109
CASP-3	Caspase-3	CCACGGAATTTGAGTCCTTCT CCACTCCCAGTCATTCCTTTAG	NM_012922.2	122
ACTB	Beta-actin	CCTAAGGCCAACCGTGAAA CAGAGGCATACAGGGACC	KJ696744.1	103

RT-qPCR was conducted with the SensiFAST SYBR No-ROX Kit on a Bio-Rad thermocycler (Bio-Rad, USA). The precise primer concentrations and PCR conditions were established during the initial optimization. Subsequent to optimization trials, tests were conducted using a total reaction volume of 10 µL, comprising equal concentrations of RNA, 5 µL of SYBR Green, 0.5 µL of each forward and reverse primer, 2 µL of ddH2O, and 2 µL of cDNA template at a concentration of 100 ng/µL. The reactions were conducted in a MicroAmp® fast 8-tube strip, covered with MicroAmp® optical 8-cap strips, under the following PCR conditions: initial denaturation at 45°C for 45 minutes, followed by 40 cycles consisting of denaturation at 95°C for five minutes, annealing at 95°C for 30 seconds, at 59°C for one minute, and extension at 72°C for 30 seconds. The concluding extension phase concluded at 72°C for a duration of ten minutes. In order to confirm the specificity of the products produced, a melting curve analysis was implemented promptly subsequent to the PCR. In order to ensure uniform amplification conditions, all samples were amplified on a single plate for each primer combination. Negative controls were implemented by substituting water for RNA templates.

The results were subsequently recorded as cycle threshold (Ct) values, and each sample was analyzed in duplicate. The comparative ΔCt method was employed to ascertain the relative expression levels of the genes in relation to the housekeeping genes for each sample. The Ct values were averaged. The ΔCt value was calculated as Ct (target gene) – Ct (ACTB), and the fold change (ΔΔCt) was calculated to quantify gene expression using the following equation: ΔΔCt = ΔCt (sample) – ΔCt (reference sample).

2.8 Statistical analysis

One-way ANOVA along with the Tukey test for post-hoc comparisons was employed to ascertain the distinctions between the control and experimental groups. The results are expressed as mean±standard error mean, with differences between groups deemed significant at $p<0.05$. Statistical analyses were conducted utilizing SPSS (version 20.0; SPSS Inc., Chicago, IL).

3. Results

3.1 Quantification of total tau protein

An ELISA kit was employed to quantify total tau, with absorbance readings acquired through a multiplate reader. The signals were subsequently transformed into ng/mL in accordance with the concentrations defined by the standard curve. Total tau protein levels in the cortex and hippocampus were measured using an ELISA assay, and the results are presented in Table 2. The STZ-LPS group exhibited a significant

Table 2. Measurement of overall tau concentrations in the cortical and hippocampal regions of the rat brain utilizing the ELISA approach.

Groups	Tau protein (ng/mL)	
	Cortex	**Hippocampus**
Control	32.48±5.76$^{\#\#\#}$	37.01±6.15$^{\#}$
MFO control	34.71±8.23$^{\#\#\#}$	38.79±12.68$^{\#}$
STZ-LPS	113.59±12.87**	77.29±3.97*
MFO 1g/kg	38.20±8.10$^{\#\#\#}$	41.78±9.23$^{\#}$
MFO 3g/kg	35.90±8.39$^{\#\#\#}$	39.03±7.64$^{\#}$

Data are presented as mean±SEM (n=6); *$p < 0.05$, **$p < 0.01$ vs. Control; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. STZ-LPS.

elevation in total tau protein levels (cortex: 113.59±12.87; hippocampus: 77.29±3.97) compared to the control (cortex: 32.48±5.76, $p<0.001$; hippocampus: 37.01±6.15, $p=0.019$) and MFO control (cortex: 34.71±8.23, $p<0.001$; hippocampus: 38.79±12.68, $p=0.027$) groups in both brain regions. Specifically, total tau levels increased by approximately 250% in the cortex and 110% in the hippocampus compared to control animals. In contrast, MFO treatment at both 1 g/kg and 3 g/kg doses significantly reduced total tau levels compared to the STZ-LPS group in the cortex (38.20±8.10, $p<0.001$; 35.90±8.39, $p<0.001$; respectively) and hippocampus (41.78±9.23, $p=0.047$; 39.03±7.64, $p=0.028$; respectively). In addition, the difference between the 1g/kg and 3g/kg MFO groups was not statistically significant ($p=0.999$), indicating that both doses effectively mitigated tau accumulation induced by STZ-LPS. Overall, the results demonstrate that MFO supplementation normalized tau levels in the cortex and hippocampus, with concentrations comparable to those observed in the control groups.

3.2 Phospo-tau-ser202 fluorescence intensity

Quantitative analysis of phospho-tau-Ser202 fluorescence intensity in the cerebral cortex of each experimental group is presented in Figure 2. The

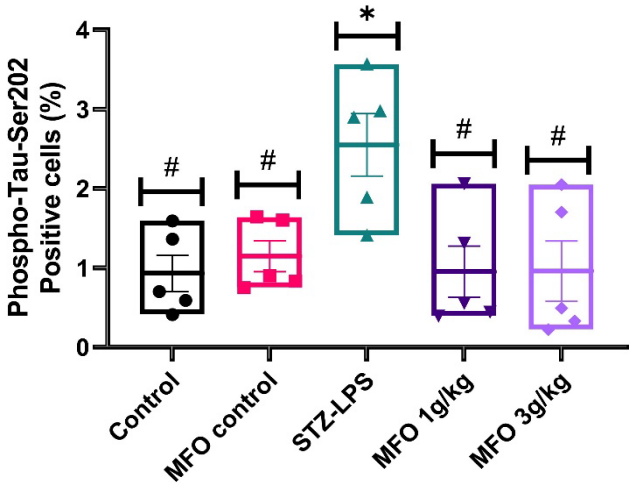


Figure 2. Quantification of phospho-tau-Ser202–positive area in the rat cortex. The bar graph shows the percentage of phospho-tau-Ser202–positive area relative to total ROI (800 × 600 pixels) across treatment groups. Data are presented as mean ± SEM (n=6), with individual data points overlaid. STZ-LPS group showed a significant increase in phospho-tau-Ser202 levels compared to controls, while MFO 1 g/kg and 3 g/kg treatments reduced this elevation. Statistical significance was determined by one-way ANOVA followed by a post hoc test. *$p < 0.05$ vs. Control and $^{\#}p < 0.05$ vs. STZ-LPS.

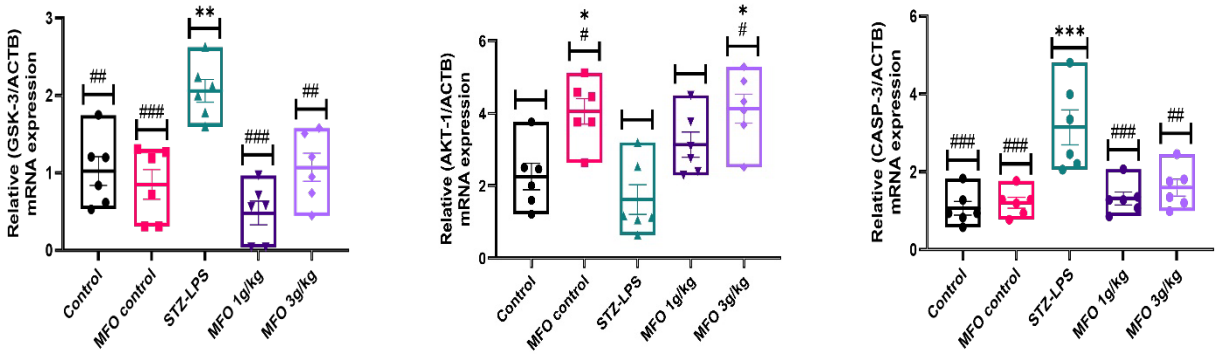
STZ-LPS group displayed a marked increase in the percentage of phospho-tau-Ser202-positive cells (2.55±0.39; p=0.013) compared to the control group (0.93±0.23), indicating enhanced tau hyperphosphorylation. Both MFO treatment groups (1 g/kg and 3 g/kg) significantly reduced phospho-tau-Ser202 levels relative (0.95±0.32, p=0.014; 0.96±0.38, p=0.014; respectively) to STZ-LPS group. There was no statistically significant difference (p=1) between the 1 g/kg and 3 g/kg MFO groups, although the 3 g/kg dose showed a slightly lower mean value.

3.3 Analysis of the relative expression of genes associated with tau phosphorylation

Figure 3 illustrates the levels of mRNA quantified through the quantitative PCR approach. The mRNA expression of genes linked to the AD pathway in the STZ-LPS group demonstrated a notable increase in GSK-3 (2.06±0.15; p=0.002) (Figure 3.A) and CASP-3 (3.15±0.45; p<0.001) (Figure 3.C) levels when contrasted with the control group (1.02±0.18 and 1.07±0.18, respectively). While the levels of AKT-1 (1.62±0.41; p=0.001) (Figure 3.B) demonstrated a reduction in comparison to the MFO control group (4.05±0.35). Conversely, the administration of fish oil 3g/kg in STZ-LPS-induced rats significantly diminishes the mRNA levels of GSK-3 (1.07±0.18; p=0.004) and CASP-3 (1.60±0.21; p=0.002), while concurrently elevating the levels of AKT-1 (4.13±0.4; p=0.001) when compared to the STZ-LPS group

4. Discussion

The STZ-LPS-induced rats in this study were able to develop tauopathy, which is considered important in AD pathogenesis. The combination of STZ-LPS induction could result in diabetic animals (range from 280-450 mg/dL), which may then promote AD development as evidenced by an increase in total tau and tau phosphorylation at serine202. In this current study also discovered that tau phosphorylation occurs via the AKT/GSK-3 pathway, as evidenced by the downregulation of the AKT-1 gene and the upregulation of the GSK-3 and CASP-3 genes. Furthermore, Wegmann and colleagues declared that phosphorylated tau proteins have been related to NFTs and tau accumulation in the brain [31]. It can be applied to differentiate AD from other forms of dementia as well [32]. Studies showed that different phosphorylation sites of the tau protein could differentiate between types of dementia. For example, phospo-tau-serine-396 distinguishes AD from chronic traumatic encephalopathy[33], phospo-tau-serine-231 distinguishes AD from vascular dementia [34], and phospo-tau-serine-181 distinguishes AD from dementia with Lewy bodies [35]. Meanwhile, total tau protein appears to be a general indicator of cortical axon damage or



A B C

Figure 3. The impact of fish oil supplementation on the relative fold change in mRNA expression across all genes among the various groups. (A) STZ-LPS significantly elevated GSK-3 expression compared to controls. MFO treatment reduced GSK-3 levels. (B) AKT-1 expression was lower in the STZ-LPS group and restored by MFO 3 g/kg supplementation. (C) STZ-LPS significantly increased CASP-3 expression, while MFO treatment (1 g/kg and 3 g/kg) attenuated this effect. Data are presented as mean±SEM; *p < 0.05, **p < 0.01, ***p < 0.001 vs. Control; $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001 vs. STZ-LPS.

neurodegeneration, as studies have revealed that total tau is increased in AD [36].

Total and phosphorylated tau protein as a biomarker for neurodegenerative diseases such as AD is mostly evaluated in clinical studies on cerebrospinal fluid (CSF) [37-38], plasma [39-40] or even tear fluid [41]. In fact, previous studies reported that total tau protein increased in AD patients evaluated from CSF [42] or plasma [43]. In the meantime, study showed that total tau and phosphorylated tau levels in CSF reflect neuropathological changes in the brain, including AD progression [44]. Meanwhile, the results of the current study are consistent with those from a previous study on AD experimental animals [45-46], which discovered changes in total tau protein and phosphorylated tau protein in the brain sample. Similar findings were also observed in an STZ-induced diabetic animal study that linked the progression of AD due to DM [9-47]. Indeed, another study reported that DM causes brain insulin impairment, which can result in abnormal hyperphosphorylation of the tau protein [48]. In addition, this abnormal hyperphosphorylation has been widely established as a result of AKT inactivation, which causes GSK-3 overactivation [49-50].

On top of everything, the result of this study demonstrated the protective effect of FO supplementation against brain tauopathy associated with DM-AD animal model. The current findings support the role of MFO in normalizing tau protein levels in both the cortex and hippocampus of STZ-LPS rats. Both 1 g/kg and 3 g/kg MFO treatments significantly reduced total tau and phospho-tau-Ser202 to levels comparable to control groups, suggesting that MFO exerts neuroprotective effects regardless of dose within this range. While a slightly greater reduction was observed with 3 g/kg MFO, the difference between the two doses was not statistically significant. It is well known that tau hyperphosphorylation at Ser202 is recognized as an early marker of neurofibrillary tangle formation [25]. While we focused on Ser202 as a representative phosphorylation site, we acknowledge that evaluating additional sites such as Ser396, Tyr18, or Thr231 would provide a more comprehensive understanding of tau phosphorylation patterns [51]. Furthermore, animal studies have mentioned that the increases in phospho-tau-serine202 occur in the diabetic brain [52] or in AD brain [53]. Moreover, research confirmed that a higher total tau level increases the risk of AD by 25% [54]. Meanwhile, tau hyperphosphorylation is undeniably described as the cause of the NFTs that leads to AD [55-56]. In line with this, research has proven a relationship between DM and tau hyperphosphorylation and the elevated risk of AD [57]. Therefore, this strengthens the argument that tau-based therapeutic strategies for AD related to DM should also consider the ability of a substance to prevent the production of tau protein as well as tau hyperphosphorylation, such as MFO administration, as demonstrated in this current study. As numerous studies have also proven the positive effect of FO on tau development and also tau hyperphosphorylation [18-58].

In this current study, the tau-related genes showed that MFO could enhance the AKT/GSK signaling pathway in STZ-LPS-induced rats as evidenced by upregulating AKT-1 genes. This activation of AKT phosphorylates GSK-3, causing it to become inactive since GSK-3 is active in resting cells and phosphorylation inhibits its activity [59], In this study, the inactive GSK-3 due to MFO was represented by a lower number of GSK-3 relative gene expression compared to the STZ-LPS group. GSK-3 inhibition will delay tau protein hyperphosphorylation and NFT aggregation, ultimately preventing cognitive dysfunction [14]. Another study reported that an insulin dysfunction, such as in DM, will block the AKT pathway and activate GSK-3, which then promotes tau hyperphosphorylation and neurofibrillary tangle [60]. On the other hand, this current study and previous study [61-62], have demonstrated that FO administration has an impact on the AKT/GSK pathway, which prevents tau hyperphosphorylation and eventually neuron apoptosis. Additionally, MFO administration in this study also demonstrated the neuroprotective ability by lowering the activity of the CASP-3 gene in rats that had been exposed to in STZ-LPS. According to Wójcik and colleagues, CASP-3 has been linked to neuronal death, learning impairment, and memory loss [63]. Although the present study could not pinpoint the specific compound within MFO responsible for these effects, previous studies have shown that omega-3 fatty acids—a key component of fish oil—can facilitate AKT translocation, leading to efficient phosphorylation and activation of AKT, inhibition of GSK-3 activity, and suppression of CASP-3 activation and subsequent cell death [64,65].

Given that MFO contains a higher concentration of EPA and DHA compared to many other omega-3 sources, it is plausible that its unique composition enhances these neuroprotective pathways, warranting further investigation.

Several limitations of the current study should be noted. First, behavioral assays, such as the Morris Water Maze or Y-maze, were not included to assess cognitive function outcomes, which limits the ability to directly correlate molecular changes with functional improvements. This limitation will be addressed in future studies, as behavioral tests are critical for confirming the translational relevance of these findings. Second, only one tau phosphorylation site (Ser202) was analyzed via immunofluorescence, and Western blot confirmation of protein-level changes for AKT, GSK-3, and CASP-3 was not performed. Additionally, normalization of RT-qPCR data was performed using ACTB without validation of reference gene stability across all treatment groups, which we recognize as another limitation. Finally, MFO was the sole omega-3 source tested, thus limiting our ability to determine which fish oil is most effective in avoiding the onset of AD-related DM. This limitation is acknowledged, and additional study is required to directly compare MFO with other omega-3 sources to clarify its advantages.

In summary, this preclinical study demonstrates that MFO supplementation mitigates tauopathy in STZ-LPS-induced rats through modulation of tau phosphorylation and regulation of the AKT/GSK-3 signaling pathway. Both 1 g/kg and 3 g/kg MFO treatments significantly attenuated tau pathology to levels comparable to controls, supporting MFO's potential as a neuroprotective dietary intervention. Future studies incorporating behavioral assessments, additional tau phosphorylation sites, and protein-level validation are warranted to further elucidate MFO's therapeutic efficacy and mechanism of action.

5. Conclusions

In conclusion, this present study demonstrates that MFO supplementation effectively mitigates tauopathy in STZ-LPS-induced rats by reducing total tau levels and tau phosphorylation at Ser202, as well as modulating the AKT/GSK-3 signaling pathway and downregulating apoptotic markers such as CASP-3. Both 1 g/kg and 3 g/kg MFO doses achieved comparable neuroprotective effects, suggesting that MFO's therapeutic impact is robust within this dosage range. These findings indicate that MFO plays a significant role in preventing neurodegeneration through the inhibition of tau hyperphosphorylation and the prevention of neuronal apoptosis. By providing molecular evidence that links omega-3 fatty acids supplementation to the regulation of tau phosphorylation and neuronal survival, this study adds to the growing body of knowledge supporting nutritional interventions in neurodegenerative disorders. Future studies should incorporate behavioral assessments, additional tau phosphorylation markers, and protein-level validations (e.g., Western blotting) to further strengthen the mechanistic understanding and translational potential of MFO as a neuroprotective agent.

Availability of Data and Materials

All data are available in this study

Author Contributions

Conceptualization, N.T., and H.A.; Methodology, N.T., and H.A.; Investigation, N.T., and A.F.; Writing – Original Draft, N.T.; Writing – Review & Editing, N.T., A.F., and H.A.; Funding Acquisition, N.T., and H.A.; Supervision, I.S.A.R., and N.S.

Ethics Approval and Consent to Participate

The Institutional for Animal Care and Use Committee (IACUC) at Universiti Putra Malaysia, Selangor, Malaysia, approved all experimental care and procedures for the animal research (Approval NO: UPM/IACUC/AUP-R017/2022).

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Wu, M., Zhang, M., Yin, X., Chen, K., Hu, Z., Zhou, Q., Cao, X., Chen, Z., & Liu, D. (2021). The role of pathological tau in synaptic dysfunction in Alzheimer's diseases. *Translational Neurodegeneration, 10(1)*, 1–11. https://doi.org/10.1186/s40035-021-00270-1.

[2] Barbier, P., Zejneli, O., Martinho, M., Lasorsa, A., Belle, V., Smet-Nocca, C., Tsvetkov, P.O., Devred, F., & Landrieu, I. (2019). Role of tau as a microtubule-associated protein: Structural and functional aspects. *Frontiers in Aging Neuroscience, 10*, 1–14. https://doi.org/10.3389/fnagi.2019.00204.

[3] Chang, H.Y., Sang, T.K., & Chiang, A.S. (2018). Untangling the tauopathy for Alzheimer's disease and parkinsonism. *Journal of Biomedical Science, 25(1)*, 1–11. https://doi.org/10.1186/s12929-018-0457-x.

[4] Aranda-Abreu, G.E., Rojas-Durán, F., Hernández-Aguilar, M.E., Herrera-Covarrubias, D., García-Hernández, L.I., Toledo-Cárdenas, M.R., & Chi-Castañeda, D. (2025). The role of Tau in neuronal function and neurodegeneration. *Neurology International, 17(5)*, 1–18. https://doi.org/10.3390/neurolint17050075.

[5] Kimura, N. (2016). Diabetes mellitus induces Alzheimer's disease pathology: Histopathological evidence from animal models. *International Journal of Molecular Sciences, 17(4)*. https://doi.org/10.3390/ijms17040503.

[6] Rad, S.K., Arya, A., Karimian, H., Madhavan, P., Rizwan, F., Koshy, S., & Prabhu, G. (2018). Mechanism involved in insulin resistance via accumulation of ß-amyloid and neurofibrillary tangles: Link between type 2 diabetes and Alzheimer's disease. *Drug Design, Development and Therapy, 12*, 3999–4021. https://doi.org/10.2147/DDDT.S173970.

[7] Bitel, C.L., Kasinathan, C., Kaswala, R.H., Klein, W.L., & Frederikse, P.H. (2012). Amyloid-ß and tau pathology of Alzheimer's disease induced by diabetes in a rabbit animal model. *Journal of Alzheimer's Disease, 32*(2), 291–305. https://doi.org/10.3233/JAD-2012-120571.

[8] Qu, Z., Jiao, Z., Sun, X., Zhao, Y., Ren, J., & Xu, G. (2011). Effects of streptozotocin-induced diabetes on tau phosphorylation in the rat brain. *Brain Research*, *1383*, 300–306. https://doi.org/10.1016/j.brainres.2011.01.084.

[9] Kim, D.H., Huh, J.W., Jang, M., Suh, J.H., Kim, T.W., Park, J.S., & Yoon, S.Y. (2012). Sitagliptin increases tau phosphorylation in the hippocampus of rats with type 2 diabetes and in primary neuron cultures. *Neurobiology of Disease, 46*(1), 52–58. https://doi.org/10.1016/j.nbd.2011.12.043.

[10] Elahi, M., Motoi, Y., Shimonaka, S., Ishida, Y., Hioki, H., Takanashi, M., Ishiguro, K., Imai, Y., & Hattori, N. (2021). High-fat diet-induced activation of SGK1 promotes Alzheimer's disease-associated tau pathology. *Human Molecular Genetics, 30*(18), 1693–1710. https://doi.org/10.1093/hmg/ddab115.

[11] Clodfelder-Miller, B.J., Zmijewska, A.A., Johnson, G.V.W., & Jope, R.S. (2006). Tau is hyperphosphorylated at multiple sites in mouse brain *in vivo* after streptozotocin-induced insulin deficiency. *Diabetes, 55*(12), 3320–3325. https://doi.org/10.2337/db060485.

[12] Zhang, Y., Huang, N. qu, Yan, F., Jin, H., Zhou, S. yu, Shi, J. shan, & Jin, F. (2018). Diabetes mellitus and Alzheimer's disease: GSK-3ß as a potential link. *Behavioural Brain Research, 339*(September 2017), 57–65. https://doi.org/10.1016/j.bbr.2017.11.015.

[13] De Simone, A., Tumiatti, V., Andrisano, V., & Milelli, A. (2021). Glycogen synthase kinase 3β: A new gold rush in anti-Alzheimer's disease multitarget drug discovery? *Journal of Medicinal Chemistry, 64*(1), 26–41. https://doi.org/10.1021/acs.jmedchem.0c00931.

[14] Sayas, C.L., & Ávila, J. (2021). GSK-3 and tau: A key duet in Alzheimer's disease. *Cells, 10*(4), 1–19. https://doi.org/10.3390/ cells10040721.

[15] Limantoro, J., de Liyis, B.G., & Sutedja, J.C. (2023). Akt signaling pathway: A potential therapy for Alzheimer's disease through glycogen synthase kinase 3 beta inhibition. *Egyptian Journal of Neurology, Psychiatry and Neurosurgery, 59*(1). https://doi.org/10.1186/ s41983-023-00751-2.

[16] Dighriri, I.M., Alsubaie, A.M., Hakami, F.M., Hamithi, D.M., Alshekh, M.M., Khobrani, F.A., Dalak, F.E., Hakami, A.A., Alsueaadi, E.H., Alsaawi, L.S., Alshammari, S.F., Alqahtani, A.S., Alawi, I.A., Aljuaid, A.A., & Tawhari, M.Q. (2022). Effects of omega-3 polyunsaturated fatty acids on brain functions: A systematic review. *Cureus, 14*(10). https://doi.org/10.7759/cureus.30091.

[17] Das, P., Dutta, A., Panchali, T., Khatun, A., Kar, R., Das, T.K., Phoujdar, M., Chakrabarti, S., Ghosh, K., & Pradhan, S. (2024). Advances in therapeutic applications of fish oil: A review. *Measurement: Food, 13*(January), 100142. https://doi.org/10.1016/j.meafoo.2024.100142.

[18] Jović, M., Lončarević-Vasiljković, N., Ivković, S., Dinić, J., Milanović, D., Zlokovic, B., & Kanazir, S. (2019). Short-term fish oil supplementation applied in presymptomatic stage of Alzheimer's disease enhances microglial/macrophage barrier and prevents neuritic dystrophy in parietal cortex of 5xFAD mouse model. *PLoS ONE, 14*(5), 1–18. https://doi.org/10.1371/journal.pone.0216726.

[19] Ma, Q.L., Yang, F., Rosario, E.R., Ubeda, O.J., Beech, W., Gant, D.J., Ping, P.C., Hudspeth, B., Chen, C., Zhao, Y., Vinters, H.V., Frautschy, S.A., & Cole, G.M. (2009). β-Amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling: Suppression by omega-3 fatty acids and curcumin. *Journal of Neuroscience, 29*(28), 9078–9089. https://doi.org/10.1523/ JNEUROSCI.1071-09.2009.

[20] Thota, R.N., Rosato, J.I., Burrows, T.L., Dias, C.B., Abbott, K.A., Martins, R.N., & Garg, M.L. (2020). Docosahexaenoic acid-rich fish oil supplementation reduces kinase associated with insulin resistance in overweight and obese midlife adults. *Nutrients 2020*, *12*(6), 1612. https://doi.org/10. 3390/NU12061612.

[21] Bibus, D. M. (2016). United States menhaden oil could save billions in U.S. health care costs and improve IQ in children. *Lipid Technology, 28*(2), 33–35. https://doi.org/10.1002/ lite.201600008.

[22] Rizzo, G., Baroni, L., & Lombardo, M. (2023). Promising sources of plant-derived polyunsaturated fatty acids: A narrative review. In *International Journal of Environmental Research and Public Health* (Vol. 20, Issue 3). https://doi.org/10.3390/ ijerph20031683.

[23] Kaur, N., Chugh, V., & Gupta, A.K. (2014). Essential fatty acids as functional components of foods - A review. *Journal of Food Science and Technology, 51*(10), 2289–2303. https://doi.org/10.1007/s13197-012-0677-0.

[24] International Fishmeal and Fish Oil Organization. (2008). The healthiest Omega-3s EPA & DHA are found mainly in fish oil and fishmeal. In International Fishmeal and Fish Oil Organization - IFFO. www.iffo.net.

[25] Neddens, J., Temmel, M., Flunkert, S., Kerschbaumer, B., Hoeller, C., Loeffler, T., Niederkofler, V., Daum, G., Attems, J., & Hutter-Paier, B. (2018). Phosphorylation of different tau sites during progression of Alzheimer's disease. *Acta Neuropathologica Communications, 6*(1), 52. https://doi.org/10. 1186/s40478-018-0557-6.

[26] Rawat, P., Sehar, U., Bisht, J., Selman, A., Culberson, J., & Reddy, P.H. (2022). Phosphorylated tau in Alzheimer's disease

and other tauopathies. *International Journal of Molecular Sciences, 23*(21). https://doi.org/10.3390/ijms23211284.

[27] Murtishaw, A. S. (2014). The effect of acute LPS-induced immune activation and brain insulin signaling disruption in a diabetic model of Alzheimer's disease. ProQuest Dissertations and Theses, 110.

[28] Arifin, W.N., & Zahiruddin, W.M. (2017). Sample size calculation in animal studies using resource equation approach. *Malaysian Journal of Medical Sciences, 24*(5), 101–105. https://doi.org/10.21315/mjms2017.24.5.11.

[29] Saputra, N.T., Suartha, I.N., & Dharmayudha, A.A.G.O. (2018). Agen diabetagonik streptozotocin untuk membuat tikus putih jantan diabetes mellitus. *Buletin Veteriner Udayana, 10*(2), 116. https://doi.org/10.24843/bulvet.2018.v10.i02.p02.

[30] Mahdi, O., Baharuldin, M.T.H., Nor, N.H.M., Chiroma, S.M., Jagadeesan, S., & Moklas, M.A. M. (2019). Chemicals used for the induction of Alzheimer's disease-like cognitive dysfunctions in rodents. *Biomedical Research and Therapy, 6*(11), 3460–3484. https://doi.org/10.15419/bmrat.v6i11.575.

[31] Wegmann, S., Biernat, J., & Mandelkow, E. (2021). A current view on tau protein phosphorylation in Alzheimer's disease. *Current Opinion in Neurobiology, 69*, 131–138. https://doi.org/10.1016/j.conb.2021.03.003.

[32] Mahaman, Y.A.R., Embaye, K.S., Huang, F., Li, L., Zhu, F., Wang, J.Z., Liu, R., Feng, J., & Wang, X. (2022). Biomarkers used in Alzheimer's disease diagnosis, treatment, and prevention. *Ageing Research Reviews, 74*(December 2021), 101544. https://doi.org/10.1016/j.arr.2021.101544.

[33] Stathas, S., Alvarez, V.E., Xia, W., Nicks, R., Meng, G., Daley, S., Pothast, M., Shah, A., Kelley, H., & Esnault, C. (2022). Tau phosphorylation sites serine202 and serine396 are differently altered in chronic traumatic encephalopathy and Alzheimer's disease. *Alzheimer's & Dementia, 18*(8), 1511–1522.

[34] Kiđemet-Piskač, S., Babić Leko, M., Blažeković, A., Langer Horvat, L., Klepac, N., Sonicki, Z., Kolenc, D., Hof, P.R., Boban, M., Mimica, N., Borovečki, F., & Šimić, G. (2018). Evaluation of cerebrospinal fluid phosphorylated tau 231 as a biomarker in the differential diagnosis of Alzheimer's disease and vascular dementia. *CNS Neuroscience and Therapeutics, 24*(8), 734–740. https://doi.org/10.1111/cns.12814.

[35] Blanc, F., & Bousiges, O. (2022). Biomarkers and diagnosis of dementia with Lewy bodies including prodromal: Practical aspects. *Revue Neurologique, 178*(5), 472–483. https://doi.org/10.1016/j.neurol.2022.03.008.

[36] Lantero-Rodriguez, J., Camporesi, E., Montoliu-Gaya, L., Gobom, J., Piotrowska, D., Olsson, M., Burmann, I.M., Becker, B., Brinkmalm, A., Burmann, B.M., Perkinton, M., Ashton, N.J., Fox, N.C., Lashley, T., Zetterberg, H., Blennow, K., & Brinkmalm, G. (2024). Tau protein profiling in tauopathies: A human brain study. *Molecular Neurodegeneration, 19*(1), 1–25. https://doi.org/10.1186/s13024-024-00741-9.

[37] Pillai, J.A., Bonner-Jackson, A., Bekris, L.M., Safar, J., Bena, J., & Leverenz, J.B. (2019). Highly elevated cerebrospinal fluid total tau level reflects higher likelihood of non-amnestic subtype of Alzheimer's disease. *Journal of Alzheimer's Disease, 70*(4), 1051–1058. https://doi.org/10.3233/JAD-190519.

[38] Visser, P.J., Reus, L.M., Gobom, J., Jansen, I., Dicks, E., van der Lee, S.J., Tsolaki, M., Verhey, F.R.J., Popp, J., Martinez-Lage, P., Vandenberghe, R., Lleó, A., Molinuevo, J.L., Engelborghs, S., Freund-Levi, Y., Froelich, L., Sleegers, K., Dobricic, V., Lovestone, S., Tijms, B.M. (2022). Cerebrospinal fluid tau levels are associated with abnormal neuronal plasticity markers in Alzheimer's disease. *Molecular Neurodegeneration, 17*(1), 1–16. https://doi.org/10.1186/s13024-022-00521-3.

[39] Dage, J.L., Airey, D.C., Wennberg, A.M.V., Hagen, C.E., Knopman, D.S., Machulda, M.M., Roberts, R.O., Jack, C.R., Petersen, R.C., & Mielke, M.M. (2016). Levels of tau protein in plasma are associated with neurodegeneration and cognitive function in a

population-based elderly cohort. *Alzheimer's & Dementia, 12*(12), 1226–1234. https://doi.org/10.1016/j.jalz.2016.06.1812.

[40] Zetterberg, H., Wilson, D., Andreasson, U., Minthon, L., Blennow, K., Randall, J., & Hansson, O. (2013). Plasma tau levels in Alzheimer's disease. *Alzheimer's Research and Therapy, 5*(2), 4–6. https://doi.org/10.1186/alzrt163.

[41] Gijs, M., Ramakers, I.H.G.B., Visser, P.J., Verhey, F.R.J., van de Waarenburg, M.P.H., Schalkwijk, C.G., Nuijts, R.M.M.A., & Webers, C.A.B. (2021). Association of tear fluid amyloid and tau levels with disease severity and neurodegeneration. *Scientific Reports, 11*(1), 1–8.

[42] Jiao, F., Yi, F., Wang, Y., Zhang, S., Guo, Y., Du, W., Gao, Y., Ren, J., Zhang, H., Liu, L., Song, H., & Wang, L. (2020). The validation of multifactor model of plasma Aß42 and total-tau in combination with MoCA for diagnosing probable Alzheimer disease. *Frontiers in Aging Neuroscience, 12*:1–9. https://doi.org/10.3389/fnagi.2020.00212.

[43] Mattsson, N., Zetterberg, H., Janelidze, S., Insel, P.S., Andreasson, U., Stomrud, E., Palmqvist, S., Baker, D., Tan Hehir, C.A., Jeromin, A., Hanlon, D., Song, L., Shaw, L.M., Trojanowski, J.Q., Weiner, M.W., Hansson, O., & Blennow, K. (2016). Plasma tau in Alzheimer disease. *Neurology, 87*(17), 1827–1835. https://doi.org/10.1212/WNL.0000000000003246.

[44] Lee, J.C., Kim, S.J., Hong, S., & Kim, Y.S. (2019). Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers. *Experimental and Molecular Medicine, 51*(5). https://doi.org/10.1038/s12276-019-0250-2.

[45] Kirk, R.A., Kesner, R.P., Wang, L.M., Wu, Q., Towner, R.A., Hoffman, J.M., & Morton, K.A. (2019). Lipopolysaccharide exposure in a rat sepsis model results in hippocampal amyloid-ß plaque and phosphorylated tau deposition and corresponding behavioral deficits. *GeroScience, 41*(4), 467. https://doi.org/10.1007/S11357-019-00089-9.

[46] Wang, L.-M., Wu, Q., Kirk, R.A., Horn, K.P., Ebada Salem, A.H., Hoffman, J.M., Yap, J.T., Sonnen, J.A., Towner, R.A., Bozza, F.A., Rodrigues, R.S., & Morton, K.A. (2018). Lipopolysaccharide endotoxemia induces amyloid-ß and p-tau formation in the rat brain. *American Journal of Nuclear Medicine and Molecular Imaging, 8*(2), 86–99. http://www.ncbi.nlm.nih.gov/pubmed/29755842%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5944824.

[47] Abdallah, H.M., El Sayed, N.S., Sirwi, A., Ibrahim, S.R.M., Mohamed, G.A., & Abdel Rasheed, N.O. (2021). Mangostanaxanthone IV ameliorates streptozotocin-induced neuroinflammation, amyloid deposition, and tau hyperphosphorylation via modulating PI3K/Akt/GSK-3ß pathway. *Biology, 10*(12). https://doi.org/10.3390/biology10121298.

[48] Guo, T., Noble, W., & Hanger, D.P. (2017). Roles of tau protein in health and disease. *Acta Neuropathologica, 133*(5), 665–704. https://doi.org/10.1007/s00401-017-1707-9.

[49] Sen, T., Saha, P., Jiang, T., & Sen, N. (2020). Sulfhydration of AKT triggers tau-phosphorylation by activating glycogen synthase kinase 3ß in Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America, 117(8), 4418–4427. https://doi.org/10.1073/pnas.1916895117.

[50] Tzeng, C.Y., Lee, W.S., Liu, K.F., Tsou, H.K., Chen, C.J., Peng, W.H., & Tsai, J. . (2022). Allantoin ameliorates amyloid ß-peptide-induced memory impairment by regulating the PI3K/Akt/GSK-3ß signaling pathway in rats. *Biomedicine and Pharmacotherapy, 153*(April), 113389. https://doi.org/10.1016/j.biopha.2022.113389.

[51] Yu, Y., Run, X., Liang, Z., Li, Y., Liu, F., Liu, Y., Iqbal, K., Grundke-Iqbal, I., & Gong, C.X. (2009). Developmental regulation of tau phosphorylation, tau kinases, and tau phosphatases. *Journal of Neurochemistry, 108*(6), 1480–1494. https://doi.org/ 10.1111/j.1471-4159.2009.05882.x.

[52] Abbondante, S., Baglietto-Vargas, D., Rodriguez-Ortiz, C.J., Estrada-Hernandez, T., Medeiros, R., & LaFerla, F.M. (2014). Genetic ablation of tau mitigates cognitive impairment induced by type 1

diabetes. *American Journal of Pathology, 184*(3), 819–826. https://doi.org/10.1016/j.ajpath.2013.11.021.

[53] Xia, Y., Prokop, S., & Giasson, B.I. (2021). "Don't phos over tau": Recent developments in clinical biomarkers and therapies targeting tau phosphorylation in Alzheimer's disease and other tauopathies. *Molecular Neurodegeneration, 16*(1), 1–19. https://doi.org/10.1186/s13024-021-00460-5.

[54] Tosto, G., Zimmerman, M.E., Hamilton, Jamie L., Carmichael, O.T., & Brickman, A.M. (2015). The effect of white matter hyperintensities on neurodegeneration in mild cognitive impairment Giuseppe. *PAlzheimers Dementia, 11(*123), 1510–1519. https://doi.org/10.1016/j.jalz.2015.05.014.

[55] Šimić, G., Babić Leko, M., Wray, S., Harrington, C., Delalle, I., Jovanov-Milošević, N., Bažadona, D., Buée, L., de Silva, R., Giovanni, G. Di, Wischik, C., & Hof, P.R. (2016). Tau protein hyperphosphorylation and aggregation in alzheimer's disease and other tauopathies, and possible neuroprotective strategies. *Biomolecules, 6(*1), 2–28. https://doi.org/10.3390/biom6010006.

[56] Xia, Y., Prokop, S., Gorion, K.M.M., Kim, J.D., Kim, J.D., Sorrentino, Z.A., Bell, B.M., Manaois, A.N., Chakrabarty, P., Davies, P., & Giasson, B. I. (2020). Tau Ser208 phosphorylation promotes aggregation and reveals neuropathologic diversity in Alzheimer's disease and other tauopathies. *Acta Neuropathologica Communications, 8*(1), 1–17. https://doi.org/10.1186/s40478-020-00967-w.

[57] Hobday, A.L., & Parmar, M.S. (2021). The link between diabetes mellitus and tau hyperphosphorylation: Implications for risk of Alzheimer's disease. *Cureus, 13*(9). https://doi.org/10.7759/cureus.18362.

[58] Scrimgeour, A.G., Condlin, M.L., Loban, A., & DeMar, J.C. (2021). Omega-3 fatty acids and vitamin D decrease plasma t-tau, GFAP, and UCH-L1 in experimental traumatic brain injury. *Frontiers in Nutrition, 8*,1–15. https://doi.org/10.3389/fnut.2021.685220.

[59] Lai, S., Wang, P., Gong, J., & Zhang, S. (2023). New insights into the role of GSK-3ß in the brain: From neurodegenerative disease to tumorigenesis. *PeerJ, 11*, 1–33. https://doi.org/10.7717/peerj.16635.

[60] Burillo, J., Marqués, P., Jiménez, B., González-Blanco, C., Benito, M., & Guillén, C. (2021). Insulin resistance and diabetes mellitus in alzheimer's disease. In *Cells* (Vol. 10, Issue 5), MDPI. https://doi.org/10.3390/cells10051236.

[61] Jia, D., Heng, L.J., Yang, R.H., & Gao, G.D. (2014). Fish oil improves learning impairments of diabetic rats by blocking PI3K/AKT/nuclear factor-κB-mediated inflammatory pathways. *Neuroscience, 258*, 228–237. https://doi.org/10.1016/j.neuroscience.2013.11.016.

[62] Sun, L.J., Hou, X.H., Xue, S.H., Yan, F., Dai, Y.J., Zhao, C.H., Wang, F., & Yang, R.H. (2014). Fish oil modulates glycogen synthase kinase-3 signaling pathway in diabetes-induced hippocampal neurons apoptosis. *Brain Research, 1574*, 37–49. https://doi.org/10.1016/j.brainres.2014.05.050.

[63] Wójcik, P., Jastrzębski, M.K., Zięba, A., Matosiuk, D., & Kaczor, A.A. (2024). Caspases in Alzheimer's disease: Mechanism of activation, role, and potential treatment. *Molecular Neurobiology, 61*(7), 4834–4853. https://doi.org/10.1007/s12035-023-03847-1.

[64] Yavari, M., Kalupahana, N.S., Harris, B.N., Ramalingam, L., Zu, Y., Kahathuduwa, C.N., & Moustaid-Moussa, N. (2025). Mechanisms linking obesity, insulin resistance, and Alzheimer's disease: Effects of polyphenols and omega-3 polyunsaturated fatty acids. *Nutrients, 17*(7), 1–23. https://doi.org/10.3390/nu17071203.

[65] Saleh, D.O., Mahmoud, S.S., Hassan, A., & Sanad, E.F. (2022). Doxorubicin-induced hepatic toxicity in rats: Mechanistic protective role of omega-3 fatty acids through Nrf2/HO-1 activation and PI3K/Akt/GSK-3ß axis modulation. *Saudi Journal of Biological Sciences, 29*(7), 103308. https://doi.org/10.1016/j.sjbs.2022.103308.