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T_3 and Curcumin Regulates Collagen-IV and Collagen-VI Expression: Role in Suppression of Amyloid-β via TGFβ1-Sp1 Signaling in Hypothyroid Mice Brain

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Abstract

Hypothyroidism has been associated with cognitive disorders and high risk of neurodegenerative disorders like Alzheimer's disease (AD), which is caused by amyloid-β (Aβ) accumulation in brain. The aim of the study is to examine the therapeutic ability of triiodothyronine (T_3) and curcumin in regulating two key extracellular matrix proteins (ECM), collagen IV and collagen VI in brain since both drugs have neuroprotective and anti-fibrotic effects. The study further explored the possible role of signaling molecules such as transforming growth factor β1 (TGFβ1) and specificity protein 1 (Sp1) in regulating the effects of T_3 and curcumin on Aβ accumulation and ECM protein expression. Experiment was carried on Balb/c mouse model by inducing hypothyroidism with 0.05% PTU followed by treatment of T3, curcumin and a combination of both. The study revealed a significant increase in the expression of collagen IV and VI, along with elevated levels of TGFβ1 and Sp1 signalling proteins, in hypothyroid conditions in response to increased Aβ expression in both the cortex and hippocampus. Western blot and RT-PCR analysis showed that T_3 and curcumin treatment downregulate the levels of ECM proteins, the APP gene, Aβ protein, and the signaling factors. The study found that T3 and curcumin treatment downregulates the collagen IV and VI expression by negatively regulating TGFβ1 and Sp1 when expression of APP and Aβ are low. T_3 and coadministration of T_3 with curcumin more effectively regulate thyroid function and ECM protein expression in both the cortex and hippocampus. Whereas curcumin alone exhibits limited impacts under the experimental conditions in our study, this may due to bioavailability, dosage or treatment duration which need to further investigation. Our results indicate a novel therapeutic approach for understanding Aβ pathology associated with hypothyroidism, emphasizing the potential association between thyroid hormone and nature-based compounds, such as curcumin, in modulating ECM dynamics and neuroprotection.

Introduction

Thyroid hormones play vital role in in brain development, neurogenesis, regulation of neurotransmitters and synaptic plasticity (Xv et al., 2024). Hypothyroidism is associated with various pathophysiological conditions in brain including dementia, impaired cognitive dysfunction and regulation metabolic processes. Several reports suggested that thyroid disorders may lead to Alzheimer’s disease (AD), characterized by amyloid plaque deposition and tau neurofibrillary tangles, and vascular dementia (Xv et al., 2024 & Chaker et al., 2016) The neuropathological markers of this disease include extracellular amyloid-beta (Aβ) plaques and the formation of intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein accumulating within the brain (Teixeira et al., 2023). However, Aβ senile plaques and NFTs are considered as the diagnostic hallmarks but the precise mechanisms that cause and accelerate AD progression remain highly unclear and under progress. People suffering with hypothyroidism have many cognitive symptoms, including memory, attention, concentration, and executing daily life functions (Li & Liu., 2024a). Further, Researchers have observed that persons with hypothyroidism had lower general intelligence, psychomotor skills, and verbal ability including a reduced hippocampus volume and reduced cerebral blood flow (Samuels MH., 2014). Therefore, it may be very

crucial for cognitive performance to keep thyroid hormone levels in the ideal range, especially for people who may be at risk for neurodegenerative diseases (Liu & Brent., 2021).

The extracellular matrix (ECM) of the brain is known to provide vital structural and functional support for brain health which includes neural growth, synaptic plasticity and the formation of neural circuits and connections (Sun et al., 2021, Gonda et al., 2020 & Soles et al., 2023). Various types of collagens such as Collagen I, II, IV and VI are present in ECM that provides mechanical support, essential for structural integrity, cell anchoring, and signal transduction mediation and regulate synaptic connectivity (Singh et al., 2023). Reports suggest that the specific localization of collagen-IV in the vascular basement membrane within the neurovascular unit (Xu et al., 2019 & Zanotti et al., 2023), has its potential involvement in clearing amyloid-beta across the blood-brain barrier by enzymatic pathway involving several proteases, including matrix metalloproteinases (MMP), which is known to be impaired in AD) Flores et al., 2023 & Weller et al., 2008). Also, collagen-VI positioned near the cell surface has also neuroprotective roles against Aβ toxicity suggest a direct interaction with neurons and thereby mitigating the damage caused by Aβ accumulation (Cescon et al., 2016, Jongsma et al., 2023 & kiuchi et al., 2002).

Transforming growth factor β1 (TGFβ1) is a pleiotropic cytokine having diverse effects within the central nervous system (CNS), including vital roles in inflammation, wound healing, and tissue repair. Dysregulation of TGF-β1 production can trigger development of AD-like pathology such as microvascular degeneration, cerebrovascular amyloidosis, and metabolic activity of brain (Dobolyi et al., 2012 & Logan et al., 1992). Reports indicate that TGFβ1 signaling pathway has both protective and potentially harmful effects in the brain which suggest the treatment strategies that target this pathway should carefully consider the disease context and stage (Su et al., 2023). Specificity protein 1 (Sp1) is a zinc finger transcription factor that binds to GC-rich areas in the promoters of many genes and it is involved in a wide range of biological processes (Kaczynski et al., 2003). As Sp1 is also associated to baseline expression of ECM genes like collagens and genes related to amyloidogenesis, it may consider an important regulator of the complicated interactions among hypothyroidism, ECM dynamics, and Aβ accumulation (Verrecchia et al., 2001 & Docagne et al., 2004).

The biologically active form of thyroid hormone, Triiodothyronine (T3) has crucial role in brain development, differentiation, other neurological activities (Bunevicius et al., 2015). T_3 restores the decreasing cognitive symptoms and reducing Aβ pathology in people experiencing AD-like symptoms under hypothyroid condition suggesting the link between hypothyroidism and an increased risk of AD (Li & Liu., 2024a & Lin et al., 2011). Thyroid hormones also regulate the expression of genes related to the remodelling of the ECM, cellular adhesion and migration, impacting the extracellular matrix's (ECM's) restructuring (Olanrewaju et al., 2024). It was observed that Hypothyroidism also leads to ECM remodelling in multiple tissues by different mechanisms (Wang et al., 2025).

Curcumin is a bioactive polyphenolic compound found in the rhizomes of the turmeric plant (*Curcuma longa L.*). It is known for its several biological roles including antioxidant, anti-inflammatory, anticancer, neuroprotective, and anti-amyloidogenic properties (Jena et al., 2012, Jena et al., 2013 & Panda et al.,

2025). It can cross the blood-brain barrier and can effectively alter several signalling pathways in the brain supports its efficacy as a therapeutic agent for neurological disorders. Although curcumin possesses great neuroprotective characters, its low bioavailability has hampered its clinical use and interest for studies on ways to increase its brain access (Genchi et al., 2024 & Razzak et al., 2019). In comparison to the use of either T_3 or curcumin individually, a combined treatment strategy seems to be more precisely control thyroid status, ECM protein expression, and Aβ pathology [Sabatino et al., 2024 & Cole et al., 2007). While both the agents show potential neuroprotective abilities, their combined effect on ECM remodelling and amyloid regulation in hypothyroid condition is unclear.

The present study is aimed to investigate the regulatory effects of triiodothyronine (T_3) and curcumin on amyloid-β expression and ECM remodelling (Collagen IV and Collagen VI) in hypothyroid mice by modulating TGFβ1 and Sp1 signaling pathway both in cortex and hippocampus. Thereby providing potential therapeutic strategies for hypothyroidism related neurological disorders.

Materials and Methods

Experimental Design

The experiment was conducted on 12to 16-week-old male Balb/c mice. Animals were housed in a colony room equipped with a constant airflow system, maintaining a controlled temperature of 23–25 °C and a 12-hour light:12-hour dark cycle. Food and water were provided ad libitum, with two mice per cage. The animals were randomly divided into six groups (N=9) as Group I: Control; Group II: PTU; Group III: PTU+T_3; Group IV: PTU+T_3+Curcumin; Group V: PTU+Curcumin; Group VI: Curcumin.

In brief, Group I: Control (animals received normal tap water throughout the experimentation, i.e., for six weeks); Group II: 6-n-propyl-2-thiouracyl (PTU)-treated mice (0.05% PTU was administered in drinking water to induced hypothyroidism in mice); Group III: Triiodothyronine (T_3) was administered to the PTU-treated mice from week four to till the end of the experiment (two weeks) designated as PTU+T_3 group; Group IV: Curcumin (30 mg/kg BW/day), doses as per Jena et al., 2012 (Jena et al., 2012) was supplemented to the mice along with T_3 to the PTU-treated animals for last two weeks known as PTU+T_3+Curcumin group; Group V: Curcumin was supplemented by oral gavaging to the PTU-treated mice for last two weeks designated as PTU+Curcumin group; Group VI: animals were administered with Curcumin only throughout the experiment as this group was called Curcumin group. The experiment was conducted for six weeks. T_3 (100µg /kg BW/day) received intraperitoneal injections of triiodothyronine dissolved in 0.01N NaOH/0.15N NaCl solution (Chaalal et al., 2019). Olive oil was served as the vehicle solution for curcumin, and the same volume was provided to the control mice. Experiments were performed in accordance with CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC) having registration number 76/GO/ReRcBi/S/99/CPCSEA dated 21.04.2022.

Behavioural Testing and Sample Collection

All animals were randomly assigned for behavioural testing in the Elevated Plus-Maze (EPM) and Open Field (OF) tasks after several days of habituation to the instruments. EPM mice (N = 6 per group) were assessed for anxiety in an elevated plus-maze (50 cm above the floor) with four arms (30 × 7 cm) connected by a common 7 × 7 cm centre area. Two opposite-facing arms were open, while the other two facing arms were surrounded by high walls (10 cm tall). Each mouse was placed in the centre, facing an open arm, and observed for 10 minutes. The maze was cleaned with ethanol after testing of each animal. The total number of entries and time spent in open and enclosed arms were counted to assess anxiety-like behaviour (Walf et al., 2007). According to Cohen et al. (Cohen et al., 2013), the total number of entries into the arms (open arms + closed arms) as well as the percentage of open arm entries ([open entries] / [total entries] × 100) were calculated. Furthermore, the anxiety index was calculated by using the following formula: Anxiety Index = 1 – {([Open arm time/Test duration] + [Open arms entries/Total number of entries])/2}.

The OF apparatus consists of a 50 x 50 cm chamber in which each mouse is free to explore for 10 minutes. Time spent and total entrances into the arena defined (as a 20 x 20cm zone in the centre of the device) were measured. The total distance travelled in the arena was utilized to assess locomotor activity, and time spent in the maze's outer zones (wall-hugging behaviour), which is indicative of anxiety-related behaviour (Kraeuter et al., 2019), was also recorded. Following one week of daily handling, behavioural testing was conducted between 8 a.m. and 7 p.m. A video camera was installed on the ceiling above each testing apparatus, and mice behaviour was recorded and analysed using the ANY-maze™ video tracking system and software (version 6.0).

Upon completion of the behavioural assessments, animals were euthanized, and the blood samples were collected to obtained serum for assessment of thyroid hormone profile. The brain of animals was extracted and their cortices and hippocampi were collected for subsequent molecular and biochemical assays. The whole brain of three animals was subjected for histological analysis.

Measurement of T_3, T_4, and TSH level

Serum T_3, T_4, and TSH level was measured, approximately 12 hours following the previous T3 or vehicle injection. The collected blood samples were subjected to centrifugation at 1000 xg for 15 minutes to obtained serum and kept at -20°C until analysis. Total T_3, T_4, and TSH in serum were measured using an ELISA Complete Kit in accordance with the manufacturer's instructions.

Real-time quantitative PCR

RNA was extracted from cortex and hippocampus (N=6) using RNA extraction kit (Trizol reagent, Invitrogen, France) and the procedure followed as per the manufacturer's instruction. The quality and quantity of RNA were evaluated using spectrophotometry. SuperScript II reverse transcriptase was used to synthesize cDNA from OligodT and random primers (Promega, France). The real-time polymerase chain reaction (PCR) was carried out in a Light Cycler system (Bio-Rad, France) with a qPCR Advanced TM SYBR® Green Supermix. The primer information for TRα1, APP695, COLIVα1, COLVIα1, TGFB1, SP1,

and GAPDH were shown in Table 1. The increased fluorescence signal from the samples is due to binding of SYBR® Green dye to double-stranded DNA at the end of the elongation stage. The intensity was measured to determine quantification. The threshold cycle (Ct) was calculated for each sample. The results were presented as a ratio of the concentration of the targeted genes to that of GAPDH (house gene) as internal control.

Western blotting

Cortical and Hippocampal tissue samples (N=6) were homogenized in ice-cold Western blothomogenization buffer. The lysates were centrifuged at 12,000 × g for 15 minutes at 4 °C, and the supernatants were collected for protein estimation using the Bradford assay. Samples were prepared using loading buffer for SDS-polyacrylamide gels (8–12%) for electrophoresis. Proteins were then transferred to polyvinylidene fluoride (PVDF) membranes (0.2 µm; Millipore). Then the membranes were blocked with 5% skimmed milk in TBST for 1 hour, and after washing, the membrane was incubated overnight at 4 °C with primary antibodies such as GAPDH (Santa Cruz Biotechnology, 1:1000), Collagen VI (Santa Cruz Biotechnology, 1:1000), Collagen IV (Novus Biologicals, 1:10000), TGF β1 (Santa Cruz Biotechnology, 1:750), β-Amyloid (Santa Cruz Biotechnology, 1:1000), and Sp1 (Santa Cruz Biotechnology, 1:1000). After washing, membranes were incubated with respective HRP-conjugated secondary antibodies (anti-rabbit or anti-mouse IgG, Santa Cruz Biotechnology, 1:5000) for 1 hour at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL; Santa Cruz Biotechnology) and quantified by densitometric analysis using ImageJ software 1.54f. Relative protein expression was normalized to GAPDH.

Histological analysis

Whole brain was fixed (N=3) using 10% Neutral buffer formalin (NBF) for a minimum of 48 hours. Following washing, they were imbedded in paraffin blocks and dehydrated in alcohol. For histological analysis, tissue sections that were 7 µm thick were stained with haematoxylin and eosin stain. The sections were observed under light microscope for qualitative analysis of nuclei and cytoplasm of control and treated groups.

Statistical analyses

Normality of the data were assessed by using Shapiro-wilk test for further statistical test. Normally distributed behavioural and biochemical data were analysed using one-way or repeated-measures ANOVA, with Tukey's post hoc tests. Values are presented as mean ± SEM. Statistical analyses were performed using GraphPad prism 8.0.1 software. p values less than 0.05 were considered statistically significant.

Results

Body weights and Brain weights

The initial and final body weights of individual mice of each group were taken and the control groups were compared with the treated groups (Table 2). The PTU-treated mice (group II) exhibited a significant reduction in body weight compared to the control animals ($F(5,12) = 5.86$, $p < 0.05$). The reduced body of group II mice were augmented up to control level on administration of T_3 alone (group III) or in combination with curcumin (group IV). However, an unchanged body weight was observed in group V mice as compared to group II. Curcumin treatment to the normal mice (group VI) showed an unaltered body weight as compared to control (group) mice. The brain weights showed no significant differences between control and other treated groups.

Behavioural consequences

In the Elevated Plus-Maze (EPM) test **(Figure: 1 a & b),** PTU-induced hypothyroid mice (group II) displayed a significant increase in anxiety-like behaviour, reflected by a higher anxiety index compared to control (group I) animals ($F(5,6) = 2.16$, $p < 0.05$). Administration of T_3 (group III), curcumin (group V) or combinatorial treatment of T_3 and Curcumin (group IV) to the hypothyroid mice showed significant reduction in anxiety index relative to PTU-treated mice (group II). Curcumin supplementation to the normal mice (group VI) did not induce anxiety and its level is similar to that of control (group I) mice ($p < 0.05$).

In the Open Field (OF) Test **(Figure: 1 c & d),** PTU-treated mice (group II) spent significantly more time in the peripheral zone compared to controls ($F(5,18) = 3.40$ $p < 0.05$), consistent with increased anxiety and reduced exploratory drive. Treatment with T_3 (group III) alone or combination with curcumin (group IV) to the hypothyroid mice markedly decreased the time spent in the periphery ($p < 0.05$) as compared to PTU-treated mice (group II), indicating reduced anxiety-like behaviour. Supplementation of curcumin to PTU-treated rats (group V) showed partial improvement, which did not reach statistical significance when compared with hypothyroid mice (group II). However, curcumin administration to the normal mice (group VI) showed similar result t that of control mice.

Thyroid Hormone Status

Serum levels of T_3, T_4, and TSH in all experimental groups were shown in Table 3. PTU administration (Group II) caused a marked reduction in T_3 ($F(5,18) = 29.91$, $p < 0.001$) and T_4 ($F(5,18) = 31.91$, $p < 0.0001$) levels, along with a significant rise in TSH ($F(5,18) = 121.6$, $p < 0.001$) compared to the control group, confirming hypothyroidism. Treatment with T_3 (Group III) and T_3 plus curcumin (Group IV) significantly restored T_3 and T_4 levels toward normal values ($p < 0.05$ and $p < 0.001$ vs PTU, respectively) and TSH levels. Combinatorial treatment of PTU and Curcumin (Group V) did not produce a significant improvement over PTU alone (group II). Curcumin only (Group VI) maintained T_3 and T_4 levels comparable to controls (NS), indicating that curcumin did not alter thyroid hormone balance under normal conditions.

Transcriptional level of TRα1

Thyroid-dependent signalling was evaluated by quantifying the thyroid hormone receptor TRα1 mRNA levels **(figure; 2 a & b)** in Cortex and Hippocampus by RT-PCR**.** T_3 target genes are typically considered as biomarkers of thyroid hormone status in tissues. One-way ANOVA analyses comparing the six groups revealed the effect of T_3 status on TRα1 ($F(5, 12) = 8.882$) on TRα1 mRNA levels. After six weeks of PTU treatment, decrease in TRα1 mRNA levels was observed in group II as compared to control ($p < 0.001$) in the cortex and hippocampus. Administration of T_3 and curcumin independently or in combination to the hypothyroid mice (group III, IV and V), the mRNA level of TRα1 in both the tissues enhanced significantly with respect to PTU-treated mice (group II). of the data indicate that T3 administration significantly enhanced the reductions in T_3 cellular actions in the cortex and hippocampus associated with hypothyroidism. Observing the experimental results, it can be expressed that curcumin alone does not effectively restore thyroid status in hypothyroid animals.

Quantitative assessment of APP695 mRNA & Aβ (1-42) peptide

Adult onset of hypothyroidism can lead to amyloid precursor protein processing of amyloidogenic pathway. As expected PTU treatment led to an increased expression of APP695 mRNA**(Figure 3; a & c, Figure 4; A &B)** level in cortex ($F(5, 12) = 89.57$, $p < 0.001$, post hoc PTU vs control, $p < 0.001$) and in hippocampus ($F(5, 12) = 191.5$: $p < 0.001$; post hoc PTU vs control: $p < 0.001$)**,** as well as western blot analysis also showing an increased level of Aβ1-42 peptide **(Figure 3; b & d, Figure 4; A &B)** ($F(5,12) = 15.23$: $p < 0.001$, post hoc PTU vs control, $p < 0.05$) in the cortex and ($F(5,12) = 15.19$; $p < 0.001$, post hoc PTU vs control, $p < 0.05$) in hippocampus. Administration of T_3 alone or in combination of T_3 and curcumin to the PTU-treated mice in group III and IV is significantly reduced the APP695 mRNA level as compared the PTU-treated mice (group II) in cortex and hippocampus (post hoc PTU+T3 vs PTU: $p < 0.001$; post hoc PTU+ T3+Curcumin vs PTU: $p < 0.001$). The expression of Aβ1-42 is significantly reduced to normal level in both group III and IV with respect to group II in both the tissues of brain (post hoc PTU+T_3 vs PTU: $p < 0.001$; post hoc PTU+ T_3+Curcumin vs PTU: $p < 0.001$). In Group IV, curcumin alone did not significantly reduce the levels of APP695 mRNA and Aβ1-42 peptide (post hoc PTU + Curcumin vs PTU: NS) in both cortical and hippocampal tissues. The group treated solely with curcumin (Group VI) exhibited an unchanged level of APP695 mRNA and Aβ1-42 peptide expressions in comparison to the control group. These data indicate that T_3 administration significantly reduces the formation of APP695 gene transcript and Aβ1-42 expression in the cortex and hippocampus associated with neurodegeneration.

mRNA & protein levels of Collagen IV and Collagen VI

RT-PCR and Western blot was done to quantify the transcript and translate level for the extracellular matrix protein Collagen IV **(Figure 3; e, f, g, h & Figure 4; A &B)** & Collagen VI **(Figure 3; i, j, k, l & Figure 4; A &B)** both in cortex and hippocampus of different experimental groups. In group II PTU treatment leads to significant upregulation of both COLIVα1 and COLVIα1 in the cortex (COLIVα1: $F(5,12) = 124.00$, $p<0.001$; COLVIα1: $F(5,12) = 135.5$, $p<0.001$) and in hippocampus (COLIVα1: $F(5,12) = 75.72$, $p<0.001$; COLVIα1: $F(5,12) = 249.1$, $p<0.001$) compared to controls. A similar pattern was observed at the protein

levels of Collagen IV and Collagen VI showing significant increases in both cortex and hippocampus ($p<0.001$). Administration of T3 significantly decreased the PTU-induced changes, restoring both Collagen IVα1 and Collagen VIα1 mRNA and their respective protein expression to near-control levels ($p<0.001$) in group III. Combined treatment of T3 and curcumin (Group IV) produced a comparable reduction in ECM genes and protein expressions with no significant difference compared to T3 only (NS). In contrast, in Group V curcumin treatment with PTU did not show a significant improvement over PTU. Curcumin-only treatment (Group VI) maintained ECM protein and gene expression similar to controls (NS).

mRNA & protein levels of TGFβ1 and Sp1

RT-PCR and Western blot was done to quantify the transcript and translate level respectively for the extracellular matrix protein associated regulatory factor TGFβ1**(Figure 4; a & b, A &B** and Sp1 **(Figure 4; c & d, A &B** in the cortex and hippocampus of different experimental groups. Expression of TGFβ1 and Sp1, two key transcriptional regulators of ECM synthesis, followed a trend consistent with that of Collagen IV and VI, where PTU-treated mice (Group II) exhibited a significant elevation in cortical (TGFβ1: $F(5,12) = 23.83$, $p<0.001$; Sp1: $F(5,12) = 14.41$, $p<0.001$) and hippocampal (TGFβ1: $F(5,12) = 52.01$, $p<0.05$; Sp1: $F(5,12) = 17.25$, $p<0.001$) expression compared to control. Corresponding elevations were also observed in TGFβ1 and Sp1 protein levels ($p<0.001$) in both cortical and hippocampal tissue. Administration of T_3 alone or in combination with curcumin to the PTU-treated mice of group III and IV was shown significantly reduced ($p < 0.05 – 0.001$) the transcript and translate level of TGFβ1 and Sp1. Although combined treatment of T_3 and curcumin (Group IV) reduced their expression compared to PTU-treated mice, but showed no additional benefit over T_3 alone (group III). Supplementation of curcumin to the PTU-treated mice (Group V) failed to significantly altered with respect to PTU-treated mice (group II). On the other hand, curcumin alone supplementation to the normal mice (Group VI) maintained both TGFβ1 and Sp1 expressions comparable to control values, confirming that curcumin did not adversely influence normal ECM turnover.

Histological outcomes

Haematoxylin and eosin staining demonstrated significant histological changes in cortex and hippocampus **(figure 5; A & B)** in treated groups compared to controls. Control mice (group I) showed normal histological structure of neurons stained blue or violet and cytoplasm-stained pink in the cerebral cortex and the hippocampus. Normal cell structure was seen in the control sections, which included complete neuropil, well-preserved neuronal morphology. On the other hand, in PTU treated mice (group II), neuronal shrinkage, hyperchromatic and pyknotic nuclei, vacuolation, and disruption of the neuropil, decreased migration of neurons was observed compared to the controls. The neurons appeared to be clustering together especially in hippocampus region in group II while in the controls normal position of neurons were observed**.** Many of these deformities were mitigated by T_3 supplementation, such as decreased neuronal pyknosis, enhanced cellular integrity, and a partial restoration of cortical and hippocampal layer architecture in group III. The most observable neuroprotective impact was seen when

T_3 and curcumin were administered together. The morphology of cortical and hippocampus in group IV, which included compact cellular layers and distinct neuronal profiles, was very similar to the control group. Although the degree of restoration was less detectable with supplementation of curcumin along with PTU in group V. Animals treated only with curcumin (group VI) showed normal histoarchitecture and no signs of neuronal damage.

Discussion

Thyroid hormone deficiency has been increasingly associated with increased susceptibility to neurodegenerative processes where anxiety and behavioural abnormalities frequently precede overt cognitive loss along with emotional disorders (Quinlan et al., 2020). The present study demonstrates that PTU-induced hypothyroidism leads to systemic and coordinated alternations in amyloid expression, ECM processing and associated signaling factors in cortex and hippocampus. PTU-treated mice showed significant weight loss and elevated TSH with reduced T_3 and T_4, confirming effective state of hypothyroid induction. These observations are compatible with the previous studies in rodent models (Vasilopoulou et al., 2016 &Taheri et al., 2018). T_3 supplementation with or without curcumin restored the serum T_3 and T_4 level toward normal and reduced TSH to control levels. On the other hand, supplementation of curcumin did not influence the altered thyroid hormone status, and remained essentially hypothyroid state. However, the study of Papiez (2023) indicated that administration of curcumin to the hypothyroid rats results in increased serum FT_4 level as compared to the hypothyroid rats (Papiez M.A., 2023). This may be due to the physiological response of specific organisms in response to different dose and duration of the treatment.

The observed behavioural patterns in PTU-induced hypothyroid mice showing a visible anxiety-like behaviour and decreased exploratory activity. In both the OFT and EPM tests, the hypothyroid mice spent significantly more time in the peripheral zone and open arm indicating increased anxiety and reduced exploratory drive (**Figure. 1, a and b**). Administration of T_3 and curcumin singly or in combination to the hypothyroid mice showed a markedly decreased time spent in the peripheral zone and open arm, indicating reduced anxiety-like behaviour. Similar pattern was also observed in hypothyroid mice treated with curcumin. This depicts the crucial role of thyroid hormone and curcumin that play in preserving emotional and cognitive balance and effectively restores normal behavioural responses. These findings are corroborated with the study of Cheng et al (2025), where they have shown hypothyroidism is associated with anxiety-like behavior in mice (Cheng et al., 2025). Curcumin which is known for its antioxidant, anti-amyloidogenic properties also reduce anxiety like conditions in animal models (Hang et al., 2024 & Abu-Taweel et al., 2021).

At molecular level, APP695 mRNA and $A\beta_{1-42}$ peptide were significantly upregulated in cortex as well as in hippocampus relative to controls in hypothyroid condition. Studies have revealed that there is a possible relationship between thyroid dysfunction and Aβ expression which is evident from enhanced levels of Aβ42 production with the increasing of APP gene expression particularly in hippocampus of

PTU-induced hypothyroid rats (Ghenimi et al., 2010 & Chaalal et al., 2014). Further, clinical and preclinical evidence has long linked hypothyroidism with increased Aβ and dementia risk (Bunevicius et al., 2015). In the current investigation, it was observed that the amyloidogenic changes in the expression of APP695 (mRNA) and Aβ42 (peptide) in hypothyroid mice were reversed by T_3 treatment alone or coadministration with curcumin. Belakavadi et al. (2011) showed that T_3 directly suppresses APP transcription via thyroid receptor binding to a negative response element in the APP promoter (Belakavadi et al., 2011). Similarly, Contreras-Jurado and Pascual (2012) noted that T_3 has a role in reversing the expression of brain APP level in hypothyroid rodents. that hypothyroidism in rodents is associated with increased brain APP levels (with T3 reversing this) (Contreras-Jurado & Pascual., 2012). AlAnazi et al. (2023) found that hypothyroidism increases Aβ production and tau hyperphosphorylation, which impairs synaptic plasticity and memory (AlAnazi et al., 2023).

THs have crucial role in brain development and maintenance, where it promotes ECM synthesis during the process of brain developmental such as neurogenesis and migration. Collagens are one of the major components of the extracellular matrix (ECM), among which Collagen IV and VI are essential for maintaining blood-brain barrier (BBB) integrity and structural stability (Khandelwal et al., 2025). In this study, PTU-induced hypothyroid brain also elevated the expression of ECM collagens, collagen IVα1 and VIα1 mRNAs as well as their proteins as compared to controls. Increased collagen expression in hypothyroid brain showed a parallel rise in Aβ expression, and further accompanied by upregulation of TGF-β1 and its transcriptional mediator Sp1.In response to T_3 treatment both collagen IV/VI and TGF-β1/Sp1 expression are reversed to normal level, consistent with T_3-driven suppression of signaling pathway (Alonso-Merino et al., 2016). It has been reported that collagen IV is localized in senile plaques, and thereby it inhibits Aβ fibrillization (Kiuchi et al., 2002). Collagen VI has a similarly role in the brain's response to Aβ (Cescon et al., 2016). In this context, Cheng et al. (2009) revealed that neuronal collagen VI is upregulated in response to Aβ accumulation via TGF-β1 signaling (Cheng et al., 2009). Further they indicated that collagen VI itself binds Aβ oligomers to prevent their toxic effects. Thus, the concurrent upregulation of collagens IV/VI and Aβ in our PTU mice may reflect a compensatory response rather than a deleterious process.

Increasing evidence suggests that TGF-β1 has a neuroprotective role against Aβ toxicity both *in vitro* and *in vivo* models of AD (Caraci et al., 2012 & Dang et al., 2011). Our findings showed that PTU increases TGF-β1 and Sp1 in cortex and hippocampus which is parallel to Aβ and ECM expression. Prior study also indicates that TGF-β is a direct stimulator of collagen production associated with sp1 transcription factor (Feng et al., 2023). That may be the elevated collagens seen in hypothyroid mice could be an endogenous compensatory reaction to excess Aβ, mediated by TGF-β1-Sp1pathway as part of a neuroinflammatory cascade. Earlier reports are consistent with our findings that indicate thyroid hormone deficiency can trigger a TGF-β1-Sp1 driven protective cascade in the brain, which includes collagen overproduction and decreased amyloid pathology.

Our study also sheds light on the roles of curcumin in this context. Curcumin only (group VI) did not significantly reduce APP/Aβ or collagen levels, ECM markers and TGF-β1/Sp1expression compared to

hypothyroid controls, suggesting that its neuroprotective effects are limited. When curcumin was combined with T_3 (group IV), the therapeutic outcome was excellent but not significantly different from T_3 alone. Both treatments restored thyroid hormones and downregulated amyloid, collagen, and TGF-β1/Sp1 to similar extents suggesting anti-amyloid properties of curcumin. As per Cheng et al. (2025) treatment with curcumin in the AD model group significantly decreased Aβ plaque accumulation in both the hippocampal and cortical regions (Cheng et al., 2025). Further, curcumin can cross the blood-brain barrier (BBB) and despite having neuroprotective effects in central nervous system (Hang et al., 2024, Yu et al., 2025, Yavarpour-Bali et al., 2019), exerted limited effects in hypothyroid condition. It is possible that normalization of thyroid status by T_3 was the primary driver of recovery, with curcumin providing additional metabolic support (Jena, S., 2015). These results do not exclude the supportive role of curcumin but the impacts may be due to its dosage, bioavailability and duration of treatment, which need to be further investigation (Yu et al., 2025).

Histological findings clearly showed neuronal cytoplasmic loss and disrupted layering of neuronal cells in cortex and hippocampus of PTU-induced hypothyroid brain. Neuronal migration, the process by which neurons move from their origin to their eventual location in the brain has been identified as one of the most significant factors controlled by thyroid hormone (Jena et al., 2013, Barakat-Walter & Kraftsik, 2018) In the histological sections decreased migration of neurons were also detected in hypothyroid mice (group II) mice brain whereas mice receiving T_3 or/and curcumin showed essentially restoration of cellular deformities both in both the regions of brain. Study demonstrated that T_3 supplementation can reverse the deformities caused due to hypothyroidism (Sepúlveda et al., 2024), and the present study indicate that hypothyroidism promotes disruption of cellular structure in brain tissues leads to neuronal disorders and malfunctions but thyroid hormone supplementation potently reverses these pathological changes. According to Shteeb et al (2025) curcumin augments the AD related histopathological deformities along with recovering memory and learning ability (Shteeb et al., 2025). Administration of curcumin showed beneficial effects in maintaining the histoarchitecture in cortex and hippocampus and also showed slightly enhanced body weight in this experiment indicating improved metabolic health. However, the brain histological showed curcumin alone has only modest effect, but when combined with T_3 showed better results. Although qualitative the overall results indicate that PTU-induced hypothyroidism causes substantial hippocampal and cortical neurodegeneration, whereas T_3 and curcumin, especially when combinatorial treatment has strong protective effects on neuronal morphology.

Several limitations should be acknowledged in the current study. First, the involvement of TGFβ1-Sp1 signaling pathway is concluded from expression pattern not genetic approach. Second, only one dose and duration of curcumin were tested; higher or longer treatments might produce stronger effects on Aβ or collagens. Third, behavioural assessment for learning and memory is not included. Lastly, translating these findings to human AD remains speculative; further validation in other models and clinical studies are necessary. Despite these limitations, the data strongly link thyroid status to Aβ and ECM regulation, highlighting potential therapeutic roles for T3 and curcumin.

Conclusion

Our study demonstrates that hypothyroidism induces simultaneous dysregulation of amyloidogenic metabolism along with extracellular matrix (ECM) remodelling, associated with increased TGFβ1 and Sp1 expression in cortex and hippocampus of mice brain. Restoration of thyroid hormone levels with triiodothyronine (T3) successfully normalizes Aβ accumulation, ECM components (collagen IV and VI), and associated signalling, which are the crucial role of thyroid hormone in maintaining brain homeostasis. The parallel increased expression in ECM proteins and amyloid levels suggests that ECM processing may represent a context-dependent, potentially compensatory response to altered proteostatic conditions rather than a purely pathological and protective process. Curcumin showed minimal and independent effects and also did not significantly improve the results beyond T3 treatment. It indicates thyroid hormone restoration is the primary driver of the observed neuroprotective effects. The above findings emphasize the importance of maintaining normal thyroid function for brain health and suggest that combining thyroid hormone with nutraceuticals like curcumin could enhance neuroprotection.

Declarations

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Tables

Table 1: Details of primer sequences used in RT-PCR of the targeted genes.

Primers	Primer Sequences (5'-3')	Annealing Temperature (˚C)	Sources
TRα1	F: CAGAGGGTGGGAGCTGGT	60	Clerget-Froidevaux et al.,2004
	R: CCTGTCCAAGGGCTGGAGGGT	65	
APP695	F: AGGTGGTCCGAGTTCCCA	58	Min et al., 2017
	R: GATAACGGCCTTCTTGTCAG	57	
COLIVα1	F: CCTCCAGGTTTCCCTACTCC	61	Min et al., 2016
	R: GGCTCCATCTCTTCCACTTG	59	
COLVIα1	F: TGCCCTGTGGATCTATTCTTCG	60	Castagnaro et al, 2018
	R: CTGTCTCTCAGGTTGTCAATG	57	
TGFβ1	F: ATCCTGTCCAAACTAAGGCTCG	60	Marcus et al, 2007
	R: ACCTCTTTAGCATAGTAGTCCGC	60	
SP1	F: ACCATGAGCGACCAAGATCA	57	Shangli et al., 2013
	R: CATTGCCGCTACCCCCATTA	55	
GAPDH	F: CACCATCTTCCAGGAGCGAG	61	Castagnaro et al, 2018
	R: CCTTCTCCATGGTGGTGAAGAC	62	

Table 2: Initial and final Body weights and brain weights of the control and experimental groups. PTU and curcumin group are compared with control group and all other groups are compared with PTU group. Data are expressed as Mean ± SD (standard deviation); Statistical significance level: *, **p<0.05 and ns; non-significant.

GROUPS	Initial Body weights (Grams)	FinalBody weights (Grams)	Brain weights (Grams)
Control	26.35±0.57	27.72±0.424	0.45±0.011
PTU Treated	26.75±0.30	25.65±0.282*	0.44±0.005 ns
PTU+T_3	26.31±0.44	27.96±0.021 ns	0.44±0.005 ns
PTU+T_3 +Curcumin	26.69±0.29	26.44±0.636*	0.46±0.01 ns
PTU+ Curcumin	26.58±0.52	27.84±0.275ns	0.47±0.005 ns
Curcumin	26.76±0.27	27.20±0.502 ns	0.46±0.02 ns

Table 3: Thyroid hormone and TSH levels in blood serum in the control and experimental groups. PTU and curcumin group are compared with control group and all other groups are compared with PTU group. Data are expressed as Mean ± SD (standard deviation); FT3: free triiodothyronine; FT4: free thyroxine; TSH: thyroid stimulating hormone. Statistical significance levels: ****p<0.0001, ***p< 0.001 and *, **p<0.05.

GROUPS	fT_3 (ng/ml)	fT_4 (µg/dl)	TSH (µIU/ml)
Control	0.91±0.020	5.52±0.639	0.011±0.001
PTU Treated	0.67±0.047***	1.26±0.168****	0.020±0.0007***
PTU+T_3	0.83±0.062**	3.15±0.875**	0.010±0.0015***
PTU+T_3 +Curcumin	0.84±0.050***	3.75±0.681**	0.010±0.0005***
PTU+ Curcumin	0.70±0.036 ns	1.40±0.219 ns	0.018±0.0006ns
Curcumin	0.90±0.010 ns	4.66±0.855 ns	0.010±0.0005 ns

Figures

Fig. 1

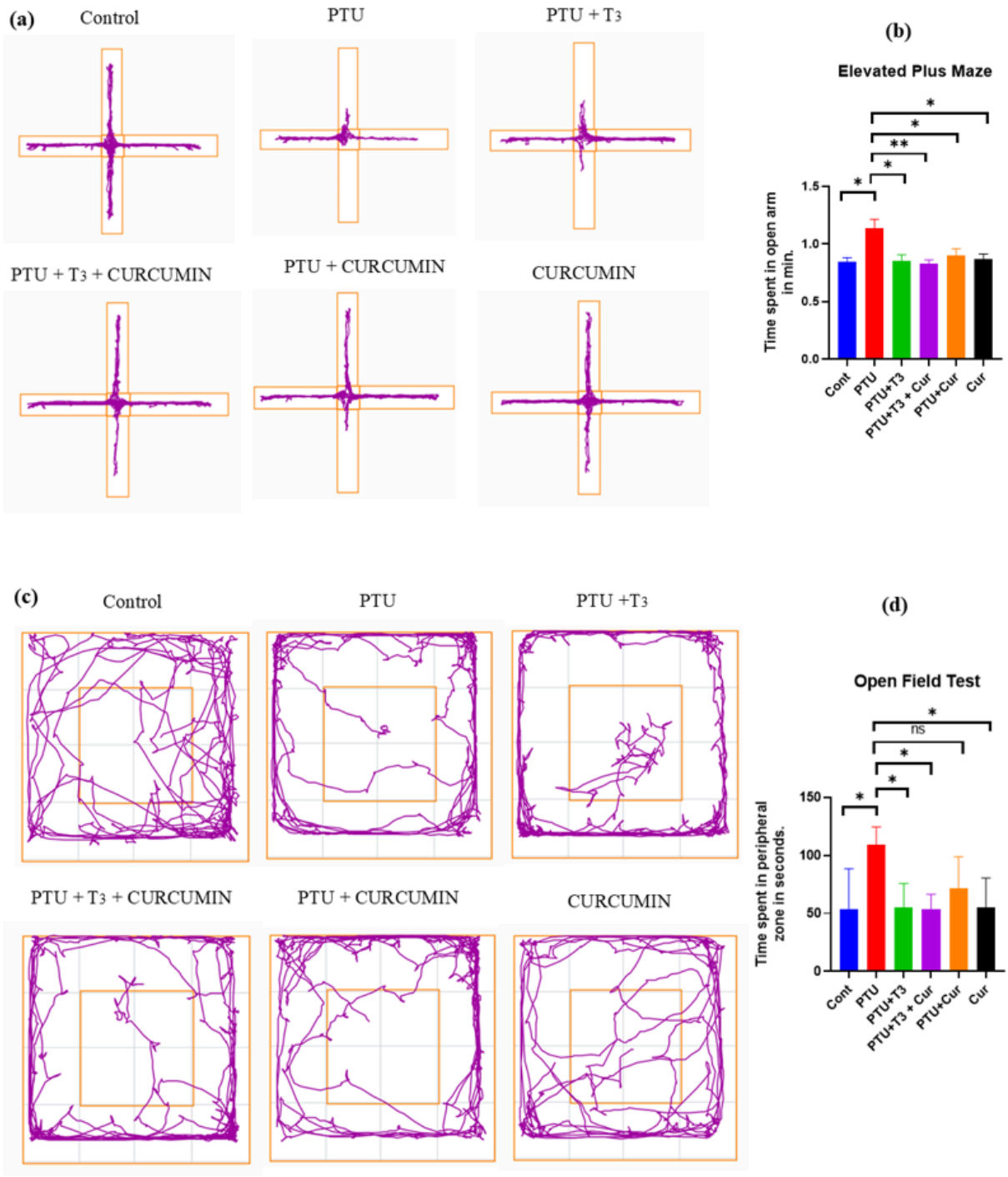


Figure 1

Behavioural Analysis of Anxiety and Locomotion in Hypothyroid and Treated Mice (a) Representative movement traces of mice in the Elevated Plus-Maze (EPM). Control animals show balanced exploration of open and closed arms, while PTU-treated mice restrict movement to closed arms, reflecting anxiety. T3 and T3+Curcumin restore open-arm exploration. (**b)** Representative movement traces in the Open Field Test. PTU-treated mice show periphery-restricted movement (thigmotaxis), while control, T3, and

T3+Curcumin groups explore the central zone. (**c)** Bar graph showing anxiety index. (**d)** Time spent in peripheral zone. Statistical notations: *p < 0.05; **p < 0.01; ***p < 0.001.

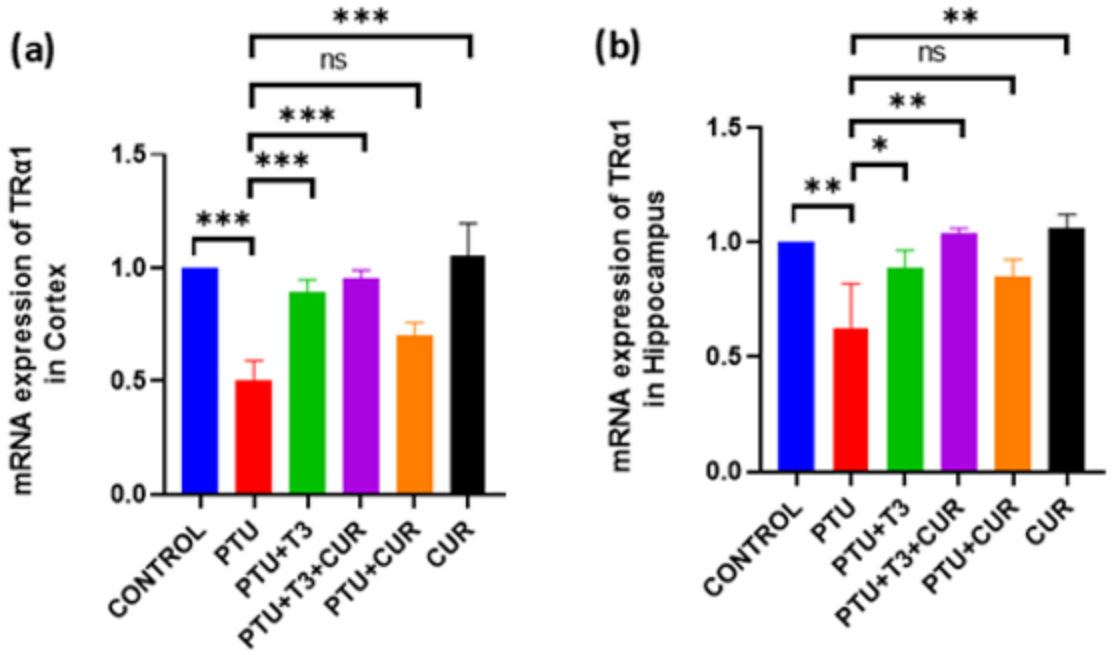


Figure 2

RT-PCR gene expression of thyroid receptor (TRα1) in Cortex (a) and Hippocampus (b) across the groups. The values were given as the mean ± SE (*n* = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey multiple comparison test at Significance level: *p < 0.05; **p < 0.01; ***p < 0.001 and ns: non-significant.

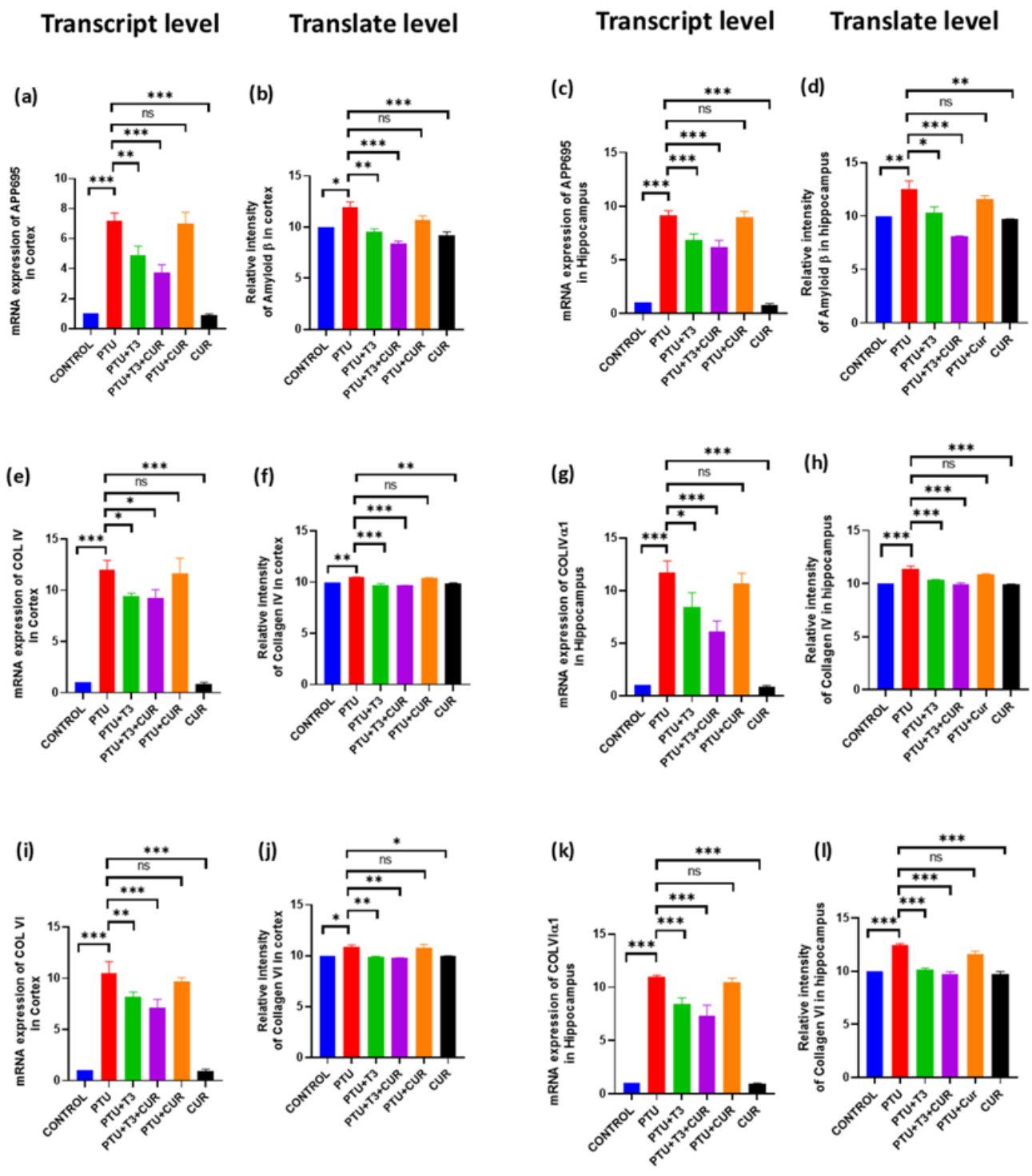


Figure 3

RT-PCR and Western blot analysis of gene and protein expressions across the experimental groups in Cortex and Hippocampus. APP695 (a & c), Aβ 1-42 (b & d), Collagen IVα1 (e & g), Collagen VI α1 (i & k), Collagen IV peptide (f & h) and Collagen VI peptide (j & l). The values were given as the mean ± SE (*n* = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey multiple comparison test at Significance level: *p < 0.05; **p < 0.01; ***p < 0.001 and ns: non-significant.

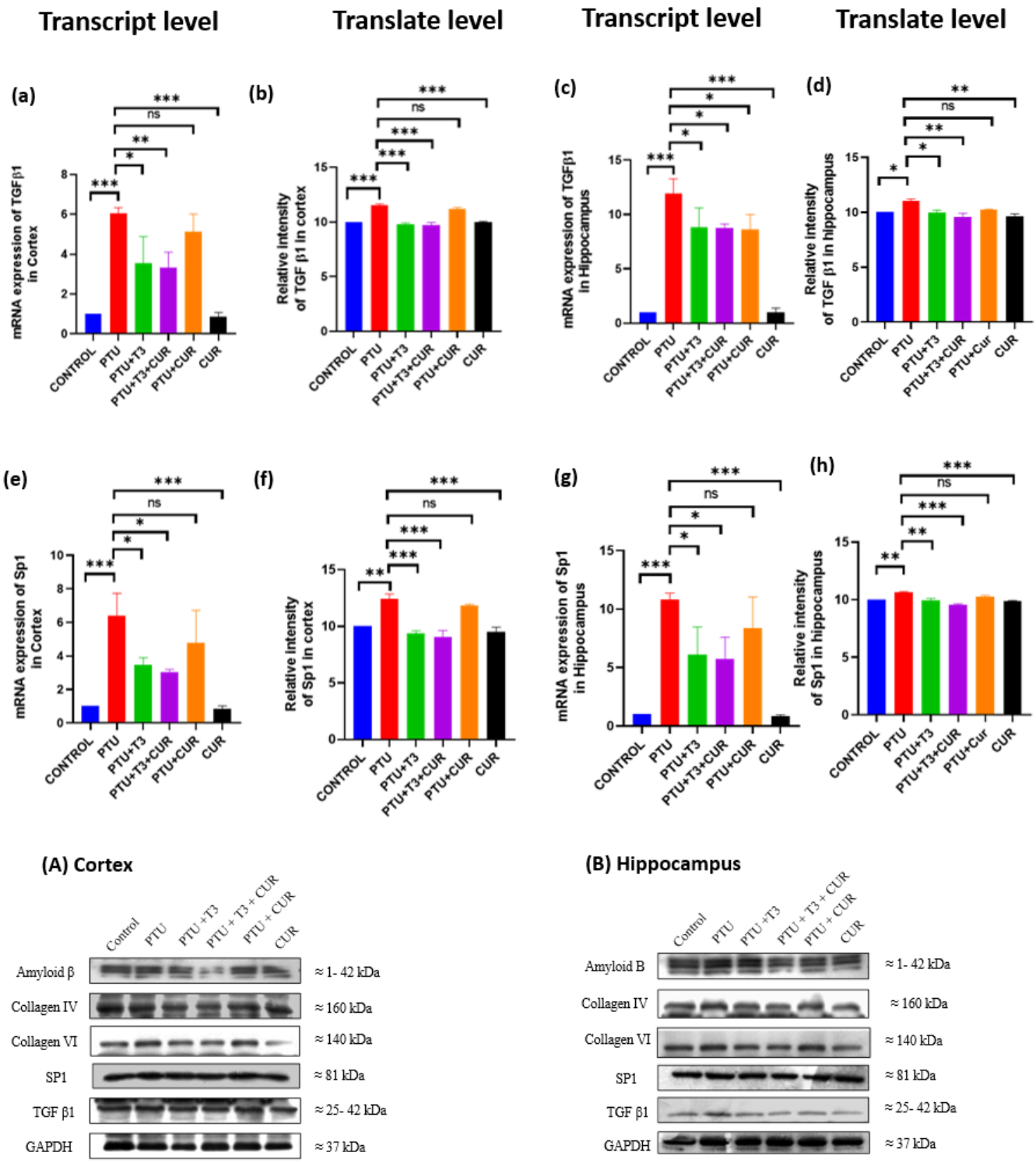


Figure 4

RT-PCR and Western blot analysis of gene and protein expressions across the experimental groups in Cortex and Hippocampus. TGFβ1 and Sp1 gene (a, c, e & g), TGFβ1 and Sp1 proteins (b, d, f & h). Representative western blot images of cortex (A) and hippocampus (B). The values were given as the mean ± SE (*n* = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey multiple comparison test at Significance level: *p < 0.05; **p < 0.01; ***p < 0.001 and ns: non-significant.

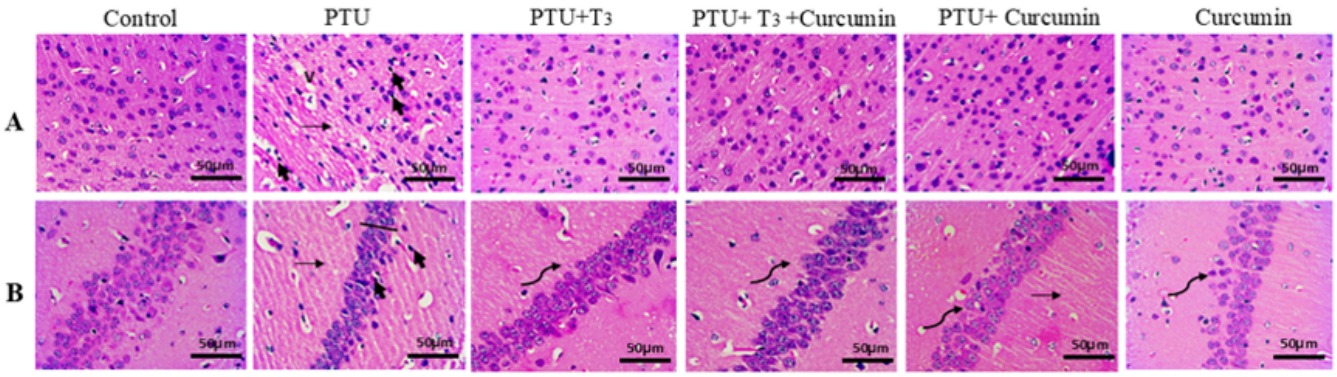


Figure 5

Histopathological examination of Cortex and Hippocampus by Haematoxylin & Eosin staining
Photomicrographic sections of the cortex (A) and hippocampus (B) of Balb/c mice of control and treated groups. Degenerating neurons with darkly stained (pyknotic) nuclei are detected (thick arrows), vacuolations (V), degradation of glial cells (normal arrows), decreased cell migration (Black bar) in PTU treated groups and restoration of cellular migration (curved arrows) in other groups are detected (H&E, ×400).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata.pdf