

Title: T₃ and Curcumin Regulates Collagen-IV and Collagen-VI Expression: Role in Suppression of Amyloid- β via TGF β 1-Sp1 Signaling in Hypothyroid Mice Brain

Sulata Kumari Sahu¹, Madhusmita Nayak^{2,4}, Achyutananda Samal¹, Sarita Jena³, Jyotsnarani Pradhan⁴, Jagneshwar Dandapat⁴, Luna Samanta¹, Srikanta Jena¹

¹Department of Zoology, CoE-Environment & Public Health, Ravenshaw University, Cuttack-753003, Odisha, India.

²Department of Botany, Government Women's college, Dhenkanal-759001, Odisha, India.

³BRIC-Institute of Life Sciences, Bhubaneswar-751023, Odisha, India.

⁴Department of Biotechnology & CoE-IOCB, Utkal University, Bhubaneswar-751004, Odisha, India.

Corresponding Author:

Srikanta Jena

Assistant professor, Department of Zoology, CoE-Environment & Public Health

Ravenshaw University, Cuttack, 753003, Odisha, India

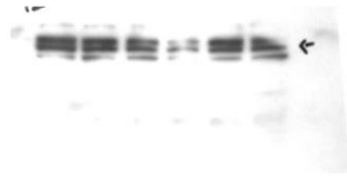
Contact: +91 9337333482

E-mail: Jenasrikanta@yahoo.co.in

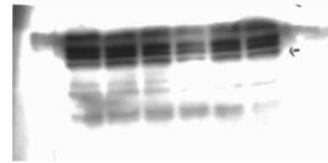
srikantajena@ravenshawuniversity.ac.in

ORCID ID: <https://orcid.org/0000-0002-3939-0646>

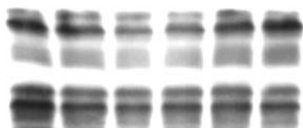
- Uncropped original western blot images



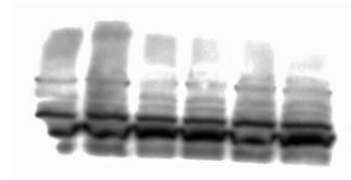
Amyloid β , 1-42kDa (Cerebral cortex)
Antibody- SC 28365
Dilution- 1:1000



Amyloid β , 1-42kDa (Hippocampus)
Antibody- SC 28365
Dilution- 1:1000



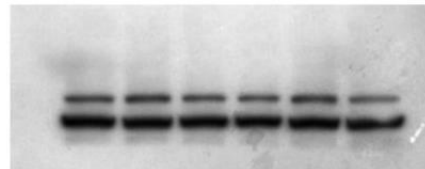
Collagen IV (Cerebral Cortex)
 $\approx$ 160 kDa
Antibody- NB120-6586
Dilution- 1:5000



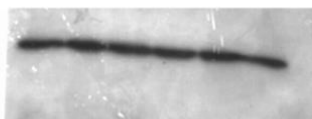
Collagen IV (Hippocampus)
 $\approx$ 160 kDa
Antibody- NB120-6586
Dilution- 1:5000



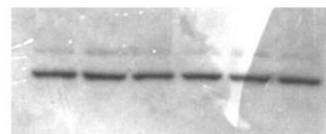
Collagen VI (Cerebral Cortex)
 $\approx$ 140 kDa
Antibody- SC 377143
Dilution- 1:1000



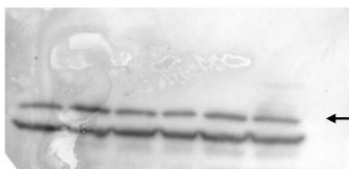
Collagen VI (Hippocampus)
 $\approx$ 140 kDa
Antibody- SC 377143
Dilution- 1:1000



SP1 (Cerebral Cortex)
 $\approx$ 81 kDa
Antibody- SC-420
Dilution- 1:1000



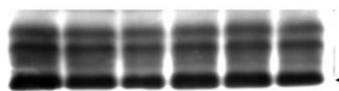
SP1 (Hippocampus)
 $\approx$ 81 kDa
Antibody- SC-420
Dilution- 1:1000



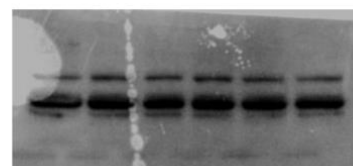
TGF- β (Cerebral Cortex)
 $\approx$ 25- 42 kDa
Antibody- SC-130348
Dilution- 1:1000



TGF- β (Hippocampus)
 $\approx$ 25- 42 kDa
Antibody- SC-130348
Dilution- 1:1000



GAPDH (Cerebral Cortex)
 $\approx$ 37 kDa
Antibody- SC-427724
Dilution- 1:1000



GAPDH (Hippocampus)
 $\approx$ 37 kDa
Antibody- SC-166545
Dilution- 1:1000

- Treatment schedule and experimental design**

	0 week	4th week	6th week
Group I	Controls	Controls	
Group II	PTU	PTU	
Group III	PTU	PTU + T ₃	
Group IV	PTU	PTU + T ₃ + Curcumin	
Group V	PTU	PTU + Curcumin	
Group VI	Curcumin	Curcumin	

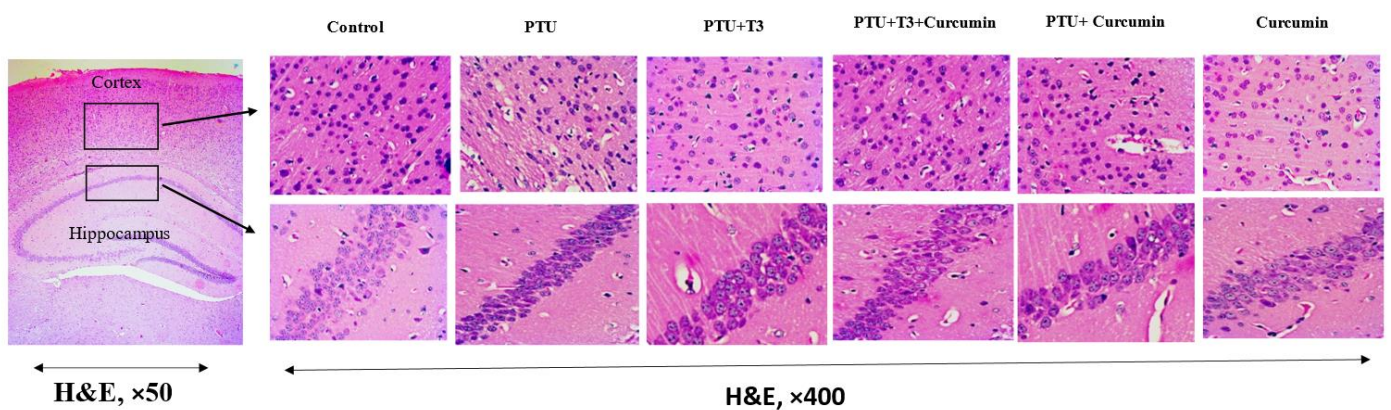
Timeline of the experimental design across the groups.

PTU- 0.05% of propylthiouracyl (6-n-propyl-2- thiouracil) dissolved in drinking water

Curcumin- 30 mg/kg BW/day dissolved in Olive oil

T₃ -100µg /kg BW/day dissolved in 0.01N NaOH/0.15N NaCl

- Photomicrographic sections of the cortex (A) and hippocampus (B) of Balb/c mice of control and treated groups with magnifications ×50 and ×400.**



- **Tables**

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

Primers	Primer Sequences (5'-3')	Annealing Temperature (°C)	Sources
TR α 1	F: CAGAGGGTGGGAGCTGGT R: CCTGTCCAAGGGCTGGAGGGT	60 65	Clerget-Froidevaux et al., 2004
APP695	F: AGGTGGTCCGAGTTCCCA R: GATAACGGCCTTCTTGTCAG	58 57	Min et al., 2017
COLIV α 1	F: CCTCCAGGTTTCCCTACTCC R: GGCTCCATCTCTTCCACTTG	61 59	Min et al., 2016
COLVI α 1	F: TGCCCTGTGGATCTATTCTTCG R: CTGTCTCTCAGGTTGTCAATG	60 57	Castagnaro et al, 2018
TGF β 1	F: ATCCTGTCCAAACTAAGGCTCG R: ACCTCTTTAGCATAGTAGTCCGC	60 60	Marcus et al, 2007
SP1	F: ACCATGAGCGACCAAGATCA R: CATTGCCGCTACCCCCATTA	57 55	Shangli et al., 2013
GAPDH	F: CACCATCTTCCAGGAGCGAG R: CCTTCTCCATGGTGGTGAAGAC	61 62	Castagnaro et al, 2018

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 $\pm$ 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 $\pm$ 0.57	27.72 $\pm$ 0.424	0.45 $\pm$ 0.011
PTU Treated	26.75 $\pm$ 0.30	25.65 $\pm$ 0.282*	0.44 $\pm$ 0.005 ^{ns}
PTU+T₃	26.31 $\pm$ 0.44	27.96 $\pm$ 0.021 ^{ns}	0.44 $\pm$ 0.005 ^{ns}
PTU+T₃+Curcumin	26.69 $\pm$ 0.29	26.44 $\pm$ 0.636*	0.46 $\pm$ 0.01 ^{ns}
PTU+ Curcumin	26.58 $\pm$ 0.52	27.84 $\pm$ 0.275 ^{ns}	0.47 $\pm$ 0.005 ^{ns}
Curcumin	26.76 $\pm$ 0.27	27.20 $\pm$ 0.502 ^{ns}	0.46 $\pm$ 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 $\pm$ 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 $\pm$ 0.020	5.52 $\pm$ 0.639	0.011 $\pm$ 0.001
PTU Treated	0.67 $\pm$ 0.047***	1.26 $\pm$ 0.168****	0.020 $\pm$ 0.0007***
PTU+T ₃	0.83 $\pm$ 0.062**	3.15 $\pm$ 0.875**	0.010 $\pm$ 0.0015***
PTU+T ₃ +Curcumin	0.84 $\pm$ 0.050***	3.75 $\pm$ 0.681**	0.010 $\pm$ 0.0005***
PTU+ Curcumin	0.70 $\pm$ 0.036 ^{ns}	1.40 $\pm$ 0.219 ^{ns}	0.018 $\pm$ 0.0006 ^{ns}
Curcumin	0.90 $\pm$ 0.010 ^{ns}	4.66 $\pm$ 0.855 ^{ns}	0.010 $\pm$ 0.0005 ^{ns}