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Appropriate Concentration of Curcumin as a Growth Factor in Neural Stem Cells

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Abstract

The growth of Neural Stem Cells (NSCs) in adult organisms is limited. Therefore, growth factors are essential to stimulate NSC proliferation and differentiation. Curcumin, a natural herbal compound, may act as a growth factor. This study promoted the growth of cryopreserved rat cortical NSCs using curcumin at varying concentrations (0.1 µM, 0.5 µM, 1 µM, and 2 µM), along with DMSO and synthetic growth factors (bFGF, TGF, and heparin) as controls. The proliferation ability of NSCs was analyzed through WST-1 assay, cell morphology, and the expression of NCS marker genes (Nestin, MAP2, and Sox2). Morphological analysis showed optimal cell reproduction at 0.5 µM. Statistical analysis using one-way ANOVA and Tukey's post hoc test on the WST-1 assay revealed significant differences between 0.5 µM curcumin and other treatment groups. Gene expression analysis indicated that Sox2, MAP2, and Nestin expression peaked at 0.5 µM. These findings suggest that the optimal concentration of curcumin to stimulate NSC proliferation was 0.5 µM. Furthermore, curcumin demonstrated comparable effects to commercial growth factors and had the potential to replace synthetic alternatives. Thus, curcumin could serve as an effective growth factor to promote the proliferation of mouse NSCs.

Keywords: NSC, Curcumin, proliferation, MAP2, Nestin, Sox2

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Introduction

Neural Stem Cells (NSCs) can repair nerve cells, thereby reducing the occurrence of abnormalities in degenerative diseases (Arundina et al., 2018; Ma et al., 2018). NSCs have emerged as a promising approach in cell-based transplants and are widely used as potential therapeutic agents for treating neurological disorders (Castelo-Branco et al., 2014; Mohammad et al., 2016). While NSCs exist in the adult brain, most remain inactive. Scientists continually refine protocols to stimulate stem cell differentiation into specific nervous system cell types for therapeutic and disease-modeling purposes (Castelo-Branco et al., 2014).

Several studies have shown that neural stem cells have limitations in neurogenesis (Udalamaththa, Jayasinghe, & Udagama, 2016). Therefore, NSC culture requires growth factors to stimulate cell proliferation and differentiation. Commercial growth factors commonly used in stem cell research are relatively expensive (Mohammad et al., 2016; Z. Chen et al., 2022). Herbal curcumin has emerged as a natural alternative growth factor due to its ability to stimulate the proliferation and differentiation of NSCs (Castelo-Branco et al., 2014).

Markers for NSC differentiation include Nestin, MAP2, and Sox2 gene expression in cells. Sox2 expression is high in embryonic stem cells and adult NSCs during proliferation and differentiation (Bott et al., 2019). Sox2 contributes to generating differentiated cells that exhibit multipotency and self-renewal capacity in the adult brain (Novak et al., 2020). Nestin is a marker found in neural stem or progenitor cells during central nervous system (CNS) development and is a filamentous protein integral to nerve cells. It serves as a marker for NSCs in both embryonic and adult brains (Bang et al., 2018). Immunofluorescence analysis has shown that all differentiated NSC cells express Nestin on progenitor cells (Z. Chen et al. 2022). The MAP2 gene plays a critical role in assembling microtubules esessential for neurogenesis. Antibodies against MAP2 serve as reliable markers for nerve cells and dendrites (Gersey et al., 2017). In this study, the researchers aimed to analyze the appropriate concentration of curcumin needed to stimulate the proliferation and differentiation of neuronal cells. This was assessed through morphological analysis, WST assays to evaluate the number of proliferating cells, and the expression of gene markers for neuronal cell proliferation, including Nestin, Cox2, and MAP2 (Bott et al., 2019; Dorn et al., 2016; Joven & Simon, 2018).

Research Methods

The research sample consisted of cryopreserved mouse cortical neural stem cells (Sigma, Cat. No. SCR029, USA) cultured using a mouse neural stem cell expansion medium (Millipore, Cat. No. SCM008, USA). The treatment involved curcumin herbal extract (Sigma, Cat. No. C7727, USA), synthetic growth factor (bFGF, EGF, and heparin), and DMSO. The research ethics permit was obtained from the Research Ethics Commission of Universitas Esa Unggul (No. 0161-21.161/dpke-kep/final-ea/ueu/vi/2021).

Neural stem cells were cultured in T75 flasks coated with Poly-L-Ornithine (Sigma Aldrich, Cat. No. P3655-10MG, USA) and laminin (Sigma Aldrich, Cat. No. CC095, USA) for two days. The flasks were first coated with Poly-L-Ornithine and re-coated with laminin at a 7 µg/mL concentration in DPBS. Poly-L-Ornithine (10 mg/mL concentration) was diluted in Aquabides and incubated overnight in a 5% CO_2 incubator at 37°C. The next day, the flasks were incubated for 2 hours in a 5% CO_2 incubator at 37°C and then stored overnight at 4^0C in a refrigerator. Before use, the flasks were incubated again for 2 hours in a 5% CO_2 incubator at 37°C.

For mouse-derived neural stem cells, the researchers used a neural stem cell basal medium (Sigma Aldrich, Cat. No. SCM014, USA) without growth factors or serum. For cell proliferation and

differentiation, the medium was supplemented with growth factors, including bFGF (Fibroblast Growth Factor) (Sigma Aldrich, Cat. No. T2815-2UG, USA), EGF (Epidermal Growth Factor) (Sigma Aldrich, Cat. No. SAB4200802 -25UL, USA), and heparin (Sigma Aldrich, Cat. No. H3149-10KU, USA).

Cells were passaged using 3 mL of Accutase solution (Sigma, Cat. No. SCR005, USA) and incubated at 37°C for 3 minutes. Subsequently, 5 mL of neural stem cell basal medium, pre-warmed to 37°C, was added to the T75 flask. The dissociated cells were transferred to a 15 mL conical tube and centrifuged at 300 ng for 2-3 minutes. After centrifugation, the supernatant was discarded, and 2 mL of neural stem cell basal medium containing growth factors was added to the cell pellet. The cell count was determined using a hemocytometer with trypan blue staining (Merck Millipore, Cat. No. CC095, USA) to assess cell viability.

The treatment consisted of curcumin herbal extract (Sigma, Cat. No. C7727- 500MG, USA) dissolved in DMSO at 0.1% (Ma et al., 2018). The curcumin treatment groups included concentrations of 0.1 μM, 0.5 μM, 1 μM, and 2 μM. The positive control used synthetic growth factors (EGF, bFGF, and heparin), while the negative control used DMSO solution without growth factors in the basal NSC medium. Following treatment, cells were incubated for 72 hours in a CO_2 incubator.

The researchers assessed cell proliferation using the WST-1 assay and the Cell Proliferation Reagent WST-1 kit (Sigma, Catalog No. 05015944001, USA). Cell cultures were seeded in a 96-well microplate at a density of $4x10^4$ cells/well in 100 μL of culture medium. After 72 hours of incubation at 37°C and 5% CO_2, 10 μL/well of WST-1 reagent was added and incubated for an additional 4 hours under the same conditions. Proliferation was measured by absorbance at 450 nm using an ELISA plate reader.

RNA was isolated using the GenElute Total RNA Purification Kit (Merck, Cat. No. RNB100, USA). Amplification of RNA samples was performed using the KAPA Probe Fast One-Step Kit (Merck, Cat. No. KK4752, USA). The primary DNA sequences used for Nestin, MAP2, Sox2, and 18S (housekeeping gene) are presented in Table 1. Data analysis included normality tests, one-way ANOVA, and Tukey HSD post hoc tests.

Table 1.
DNA Primers for Nestin, MAP2, Sox2, and 18S genes

Primer	Length	Tm
18S	20	59.04
F: 5’ TGGCAATGGTTT CGTTCTGG GGAA		
R: GCTGGCATGTAGAACC	20	59
Nestin	20	56
F: 5’ AGAGCAAGTGAA TGG GAGGA 3’		
R: 5’ GGTGAGCCACA GAAGAAAGG 3’		
MAP2	20	55
F: 5’ TGCCCTTGGGTT TAACTTTG 3’		
R: 5’ AAAGCAAGGCA TCTTCTCCA 3’		
SOX2	20	53
F: 5’ ACTGCTCTAACG CCACACCT 3’		
R: 5’ TGATATACGGC CACCACTGA 3’		

Research Results and Discussion

After thawing NSC cells from the freezing medium, the cells were cultured using NSC Complete Medium with the addition of growth factors (FGF, EGF, and heparin) from passage 0 to passage 3. The viability rate was calculated based on the number of living cells compared to the total number of cells, including dead cells (see Figure 1). The cell culture results up to passage 3 showed an increase in viable cells, resulting in higher cell viability (see Figure 2). Cell viability reached 97.48% at passage 3. Cell cultures with viability >80% were considered suitable for proliferation and differentiation tests (see Figure 2).

Figure 1
Live cells (yellow arrow) and dead cells (red arrow) cultured at the third passage. Magnification: 100x.

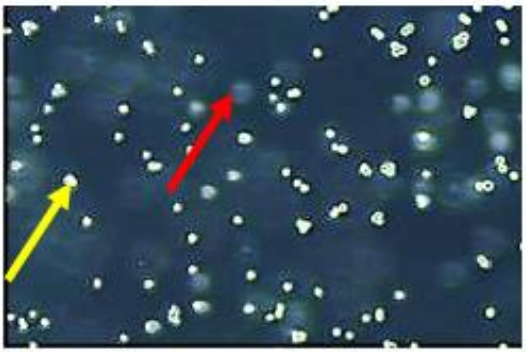
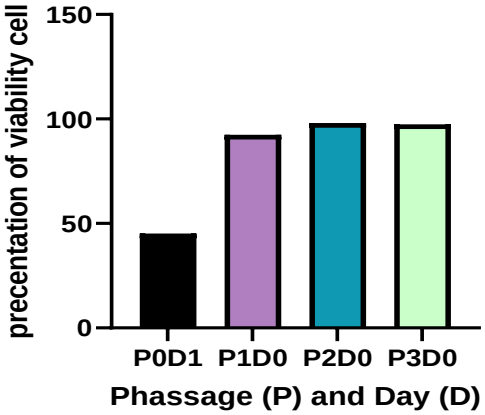


Figure 2
Percentage of cell viability from passage 0 to passage 3 on day 0.

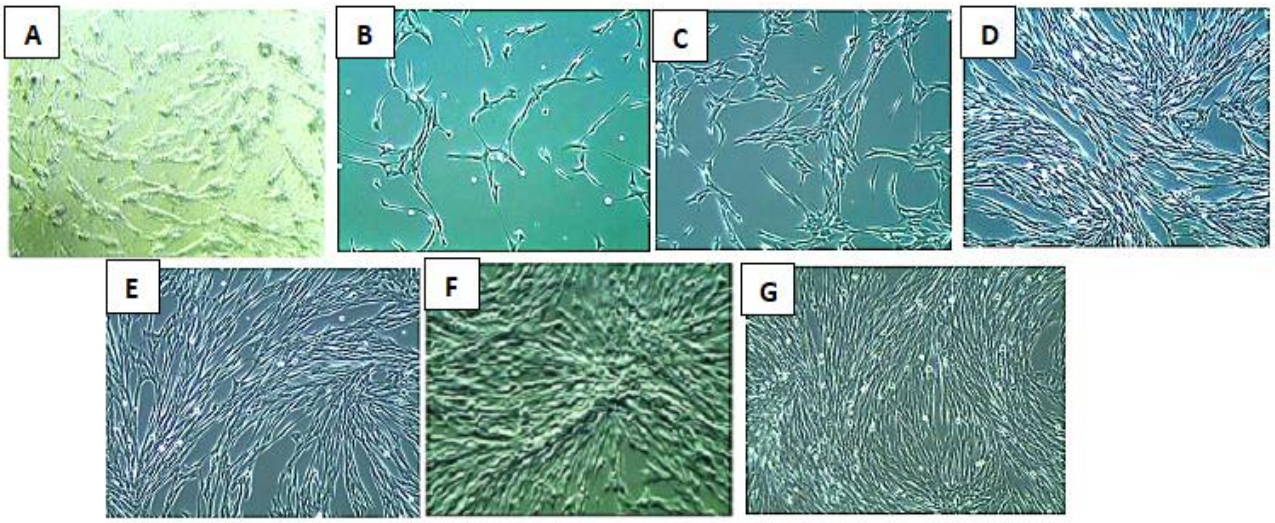


Morphological observations of cultured NSC cells using NSC Complete Medium, enriched with growth factors such as FGF, EGF, and heparin, were conducted from passage 0 to passage 2 before curcumin treatment (see Figure 3).

Observations were performed on day 3 of each passage when the cells began to differentiate into fibroblast-like cells. This differentiation progressed from passage 0 to passage 2 by the third day of each passage.

Figure 3

Morphology of cultured cells after thawing during several passages before treatment: (A) Passage 0, Day 3 (P0D3); (B) Passage 1, Day 3 (P1D3); (C) Passage 1, Day 4 (P1D4); (D) Passage 1, Day 5 (P1D5); (E) Passage 2, Day 1 (P2D1); (F) Passage 2, Day 2 (P2D2); (G) Passage 2, Day 3 (P2D3). Magnification: 100 x.

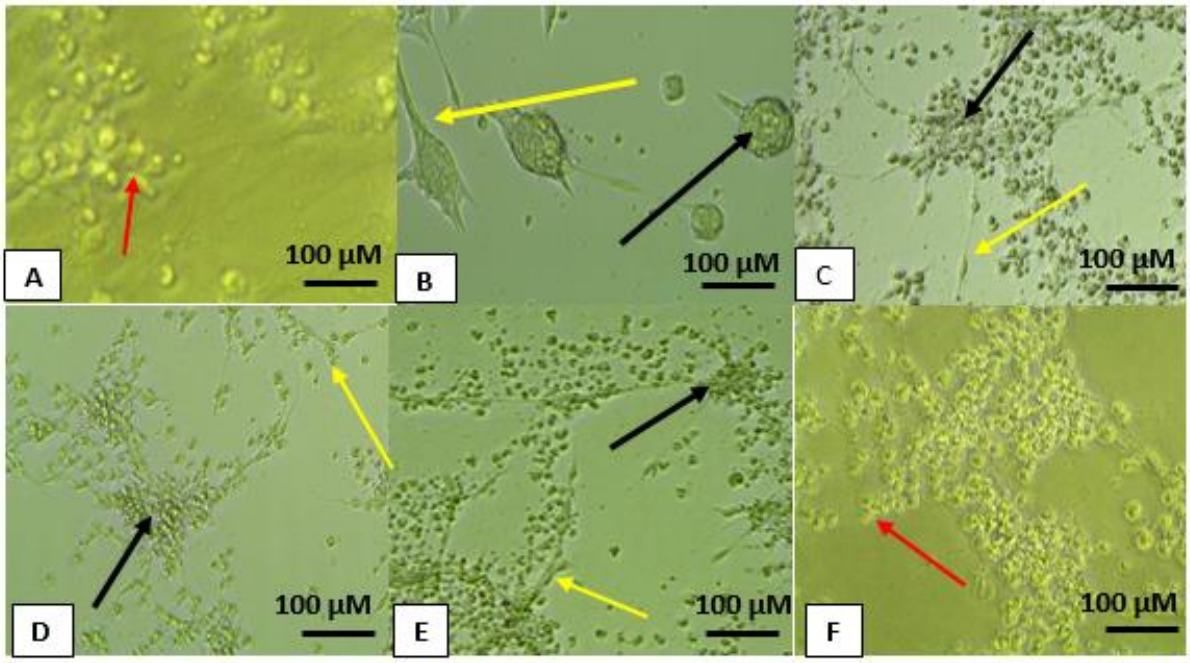


The morphology of NSC cells cultured after treatment with curcumin extract, DMSO, and synthetic growth factors is displayed in Figure 4. After achieving 97.48% viability at passage 3, the cells were treated with curcumin extract, DMSO, and synthetic growth factors. The morphology remained predominantly monocellular in the DMSO group and certain curcumin-treated groups. However, the treatment groups with 0.5 µM curcumin and synthetic growth factors exhibited denser cell growth, forming neurospheres and fibroblast-like cells. In the 0.1 µM and 1 µM curcumin-treated groups, cell growth was sparse but still displayed neurosphere and fibroblast-like cell formation (see Figure 4).

The NSC culture began with a thawing process that influenced cell growth and differentiation, followed by stimulation using growth factors. Morphological cell analysis revealed that in the positive control group, neurosphere and fibroblast-like cells were observed at the third passage and third day (P3D3). The positive control group utilized a medium enriched with growth factors such as bFGF, EGF, and heparin. EGF, a single-chain polypeptide, plays a significant role in cell proliferation and differentiation in mammals, including mouse NSC cells (Mizukoshi et al., 2016).

Cells grown in media enriched with EGF, FGF-2, or both demonstrated high levels of cell proliferation (Y. Chen, Xu, & Lin, 2017). Heparin, a mucopolysaccharide with anticoagulant properties, facilitated the binding of bFGF to its cellular receptor and enhanced EGF stability. Therefore, in the passage of mouse NSC culture, media enriched with bFGF, EGF, and heparin effectively stimulated cell proliferation and neuronal differentiation in this study (Yasunaga et al., 2022).

Figure 4
Differences in NSC cell morphology on day 3 after curcumin treatment: (A) DMSO group: monocellular morphology (red arrow); (B) Synthetic growth factor group: neurosphere (black arrow) and fibroblast-like cells (yellow arrow); (C) Curcumin 0.1 µM: neurosphere (black arrow) and fibroblast-like cells (yellow arrow); (D) Curcumin 0.5 µM: neurosphere (black arrow) and fibroblast-like cells (yellow arrow); (E) Curcumin 1 µM: neurosphere (black arrow) and fibroblast-like cells (yellow arrow); (F) Curcumin 2 µM: monocellular morphology (red arrow). Magnification: 100x (A, C-F); 400x (B).



Proliferating cells absorbed fluorescent light and appeared colored with different OD values, indicating the number of proliferating cells. The WST-1 assay provided quantitative data on the number of proliferating cells after treatment with curcumin, DMSO, and a synthetic growth factor. OD data were analyzed by calculating the stimulation index of cell proliferation (see Table 2).

Table 2.
Stimulation Index of Cell Proliferation by WST-1 Assay

Treatment	Stimulation Index of Proliferation
Positive Control	0.389937
0.1 µM	0.144037
0.5 µM	0.18629
1 µM	0.172333
2 µM	0.152264
DMSO	0.127096

The results of the WST-1 assay for analyzing the number of proliferating mouse NSC cells were measured at the third passage under treatments of 0.1 µM, 0.5 µM, 1 µM, and 2 µM. The stimulation index of cell proliferation was calculated based on the presentations of cell cultures without curcumin treatment. The Shapiro-Wilk

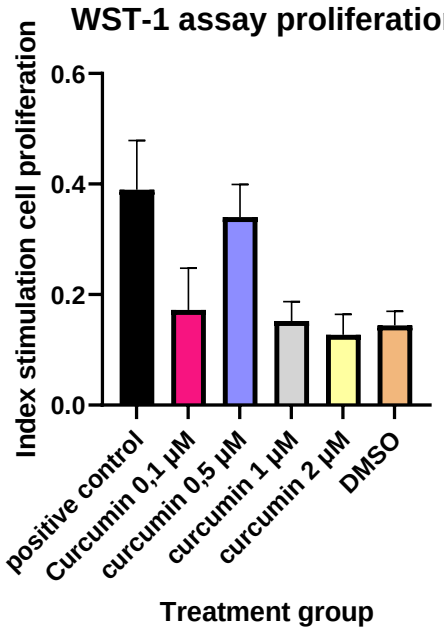
statistical test indicated that the presentation of proliferating cells was normally distributed ($p > 0.05$). The one-way ANOVA and Tukey post hoc tests showed a significant difference between the positive control group (synthetic growth factor) and the other treatment groups, except for the 0.5 µM. The 0.5 µM treatment group also demonstrated significant differences from the other treatment groups, except for the positive control group. However, no significant difference was observed between the 0.5 µM treatment and the positive control groups. The stimulation index of cell proliferation is illustrated in the bar chart (see Figure 5).

No neurosphere or fibroblast-like cell morphology was observed in the DMSO group. Singh et al. (2017) reported that low DMSO concentrations (0.01-0.001%) could increase cell proliferation when cultured in vitro on plastic surfaces for four days, whereas higher concentrations (0.5-3%) decreased cell viability (Singh, Kumar, & Singh, 2020). In addition, a 0.1% DMSO concentration is known to be non-toxic. According to Galvao et al. (2014), DMSO concentrations below 10% exhibit low toxicity (Galvao et al., 2014).

Morphological analysis revealed that neuron cells proliferated as neurospheres in the 0.5 µM curcumin treatment group. The cell density in this group was higher than in the other curcumin treatment groups. These results indicated that curcumin could stimulate the proliferation and differentiation of NSC cells. Bang et al. (2017) demonstrated curcumin's ability to stimulate NSPC proliferation at concentrations below 1 µM. However, concentrations above 5 µM reduced SC-NSPC proliferation (Bang et al., 2018). In this study, the cell morphology observed at the third passage and on the third day of treatment was the neurosphere. Neurospheres are precursors to neuron cells that eventually differentiate into adult cells (Gersey et al., 2017). According to Attari et al. (2015), this pattern was not observed after 72 hours of treatment with varying doses of curcumin (Attari et al., 2015). Neurospheres are morphologies of neuron cells that differentiate into adult neuron cells, including astrocytes, neurons, and neuroglia (Bamba et al., 2024).

Figure 5

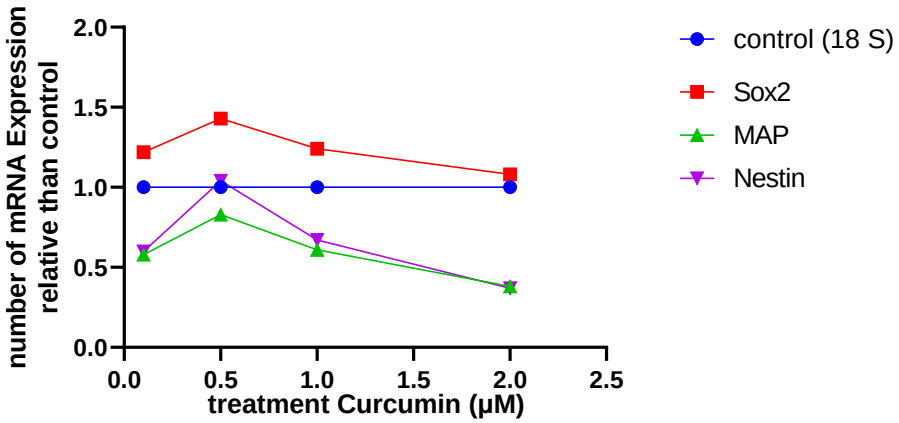
Bar chart of stimulation index of cell proliferation by WST-1 assay in mouse NSC cell culture



The Sox2, MAP2, and Nestin gene expression tests using the qPCR assay showed that the Sox2 gene expression was relatively higher than that of the control (see Figure 6). Meanwhile, the expression of MAP2 and Nestin genes was relatively lower than that of the negative control.

The proliferative stimulation index from the WST-1 assay demonstrated that the growth factor treatment group (EGF, bFGF, and heparin) had the highest index among the other treatment groups. Meanwhile, the 0.5 µM curcumin treatment group showed a relatively higher stimulation index than the other treatment groups and the DMSO group. The WST-1 assay measures the relative proliferation rate of cells based on the conversion of the WST-1 tetrazolium salt to a colored product by the mitochondrial dehydrogenase enzyme (Chamchoy et al., 2019). A more significant color change is proportional to the absorbance value, and the amount of mitochondrial dehydrogenase indicates the presence of mitochondrial activity during the cell proliferation process (Mohammad et al., 2016).

Figure 6
Graph of mRNA expression of Sox2, MAP2, and Nestin genes relative to the 18S gene (control).



A comparison of the expression levels of Nestin, MAP2, Sox2, and 18S genes in each curcumin treatment group and the positive control treatment group containing growth factors EGF, bFGF, and heparin is shown in Figure 6. In each curcumin treatment group, the MAP2 and Nestin genes exhibited lower expression than the 18S gene. The Sox2 gene expression in each curcumin treatment group, including the positive control group, appeared relatively higher than the expression of the 18S control gene, MAP2 gene, and Nestin gene.

Gene expression data were tested for normal distribution using the Shapiro-Wilk test ($p > 0.05$). One-way ANOVA and Tuckey post hoc tests revealed significant differences between the control (18S gene) and the Sox2, MAP2, and Nestin gene expression in each treatment group. Sox2 gene expression significantly differed from MAP2 and Nestin in each treatment group ($p < 0.05$). Statistical tests for the distribution of normality data on the number of proliferating cells showed a p-value > 0.05, indicating that the data were normally distributed. One-way ANOVA and Tukey post hoc test results showed that cell proliferation index values from the WST-1 assay were not significantly different between the curcumin treatment group (0.1 µM, 1 µM, and 2 µM) and the DMSO group. Significant differences were observed between the positive control and all treatment groups, except for the 0.5 µM treatment group. The positive control group and the 0.5 µM group were not significantly different.

The results indicated that curcumin could act as a growth factor stimulating the differentiation of NSC cells. Curcumin is a growth factor that can stimulate the proliferation of mouse NSCs. The herbal extract curcumin has the same effect as commercial growth factors and can be used as a substitute. The results showed that curcumin at a concentration of 0.5 µM was the most effective for promoting the differentiation process of NSCs. Attari et al. (2015) demonstrated that NSCs could proliferate at a 0.5 µM curcumin concentration after 72 hours of treatment. A study by Bang et al. (2018) reported a significant increase in the number of proliferative neural stem cells and newly formed neurons in the mouse hippocampus, along with a decrease in the number of apoptotic neurons after curcumin treatment via the Notch signaling pathway (Bang et al., 2018).

The marker gene expression (MAP2, Nestin, and Sox2) in the curcumin treatment group showed that all gene expressions peaked at a 0.5 µM concentration (see Figure 6). The results indicated that 0.5 µM curcumin was the appropriate concentration to stimulate the proliferation of mouse NSCs, as the markers of cell proliferation genes reached their peak at this concentration. Sox2 gene expression was relatively higher than that of the control gene (18S), which served as a housekeeping gene. In contrast, MAP2 and Nestin mRNA expressions were lower than the 18S gene expression. One-way ANOVA and Tukey's post hoc tests revealed a significant difference between the expression of the Sox2 gene and the expression of the other genes. Sox2 protein plays a substantial role in the central nervous system (CNS) and peripheral nervous system (PNS) by controlling the proliferation and differentiation of fetal progenitor cells. Sox2 expression is also critical for the proliferation and differentiation of neural progenitor cells in the retina (Castelo-Branco et al., 2014). SOX2, a stem cell marker, has been shown to regulate stem cell self-renewal and maintain its pluripotency (Oguchi, Iwamoto, & Higuchi, 2024).

In the research of Mohamad et al. (2016), at the maturation stage—when the neural stemness gene (NES) reached its lowest expression level—an increase in NF-L expression indicated that the cells entered the maturation stage and were no longer classified as NSCs. Besides, MAP2 expression increased at the end of this stage (Mohammad et al., 2016). The low expression of the MAP2 gene in the present research results suggested that the neuronal cell proliferation stage, from passage three on day three, had not yet reached the stage of cell maturation. Wang et al. (2017) observed Nestin and MAP2 expression using Western blot one week after spinal cord injury in Sprague-Dawley rats. Nestin was found distal to the axon and not at the dendritic ends. In this case, Nestin regulated the dynamics of neuron cell growth (Wang et al., 2017). The results of this study, showing the low expression of Nestin, indicated that the neurons being studied had not yet matured into adult neurons with axons. However, neurosphere morphology formed at a 0.5 µM curcumin dose, suggesting the development of adult neurons. The neurosphere began the formation of adult neurons (Gersey et al., 2017).

Conclusion

The appropriate concentration of curcumin for the growth of mouse NSCs (neural stem cells) was determined to be 0.5 µmol/L. At this concentration, the morphology of the neuron cells was denser and contained numerous neurospheres, indicative of adult neurons. Results from the WST-1 assay demonstrated the highest number of proliferative cells at this concentration. Additionally, the expression of markers for proliferating neuron cells, namely Sox2, Nestin, and MAP2 genes, peaked at this dose. Among these, Sox2 expression was the highest, indicating that the neuron cells at the observation time were proliferative but not yet mature. This was further supported by the relatively low expression of the MAP2 gene and the absence of axon formation, as indicated by the common expression of the Nestin gene.

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References

Arundina, Ira, Ketut Suardita, Indeswati Diyatri, and Meircurius Dwi. 2018. "Journal of International Dental and Medical Research ISSN 1309-100X Ira Arundina and et Al Volume • 11 • Number • 3 • 2018 Experimental Article." *J Int Dent Med Res*. Vol. 11. http://www.jidmr.com.

Attari, Fatemeh, Maryam Zahmatkesh, Hadi Aligholi, Shahram Ejtemaei Mehr, Mohammad Sharifzadeh, Ali Gorji, Tahmineh Mokhtari, Mojtaba Khaksarian, and Gholamreza Hassanzadeh. 2015. "Curcumin as a Double-Edged Sword for Stem Cells: Dose, Time and Cell Type-Specific Responses to Curcumin." *DARU, Journal of Pharmaceutical Sciences* 23 (1): 2703–6. https://doi.org/10.1186/s40199-015-0115-8.

Bamba, Kenzo, Midori Ozawa, Hiroaki Daitoku, and Arihiro Kohara. 2024. "Diverting the Food-Freezing Technology Improves the Cryopreservation Efficiency of Induced Pluripotent Stem Cells and Derived Neurospheres." *Regenerative Therapy* 27: 83–91. https://doi.org/10.1016/j.reth.2024.03.007.

Bang, Woo Seok, Kyoung Tae Kim, Ye Jin Seo, Dae Chul Cho, Joo Kyung Sung, and Chi Heon Kim. 2018. "Curcumin Increase the Expression of Neural Stem/Progenitor Cells and Improves Functional Recovery after Spinal Cord Injury." *Journal of Korean Neurosurgical Society* 61 (1): 10–18. https://doi.org/10.3340/jkns.2017.0203.003.

Bott, C. J., C. G. Johnson, C. C. Yap, N. D. Dwyer, K. A. Litwa, and B. Winckler. 2019. "Nestin in Immature Embryonic Neurons Affects Axon Growth Cone Morphology and Semaphorin3a Sensitivity." *Molecular Biology of the Cell* 30 (10): 1214–29. https://doi.org/10.1091/mbc.E18-06-0361.

Castelo-Branco, Gonçalo, Tobias Lilja, Karolina Wallenborg, Ana M. Falcão, Sueli C. Marques, Aileen Gracias, Derek Solum, et al. 2014. "Neural Stem Cell Differentiation Is Dictated by Distinct Actions of Nuclear Receptor Corepressors and Histone Deacetylases." *Stem Cell Reports* 3 (3): 502–15. https://doi.org/10.1016/j.stemcr.2014.07.008.

Chamchoy, Kamonwan, Danaya Pakotiprapha, Pornpan Pumirat, Ubolsree Leartsakulpanich, and Usa Boonyuen. 2019. "Application of WST-8 Based Colorimetric NAD(P)H Detection for Quantitative Dehydrogenase Assays." *BMC Biochemistry* 20 (1): 1–14. https://doi.org/10.1186/s12858-019-0108-1.

Chen, Ying, Hanqian Xu, and Gufa Lin. 2017. "Generation of IPSC-Derived Limb Progenitor-like Cells for Stimulating Phalange Regeneration in the Adult Mouse." *Cell Discovery* 3: 1–14. https://doi.org/10.1038/celldisc.2017.46.

Chen, Zheng, Lin Wang, Chichi Chen, Jie Sun, Junchao Luo, and Wenguo Cui. 2022. "NSC-Derived Extracellular Matrix-Modi Fi Ed GelMA Hydrogel Fi Brous Scaffolds for Spinal Cord Injury Repair." https://doi.org/10.1038/s41427-022-00368-6.

Dorn, Julia, Alexandra Yassouridis, Axel Walch, Eleftherios P. Diamandis, Manfred Schmitt, Marion Kiechle, Ping Wang, et al. 2016. "Assessment of Kallikrein-Related Peptidase 5 (KLK5) Protein Expression in Tumor Tissue of Advanced Ovarian Cancer Patients by Immunohistochemistry and ELISA: Correlation with Clinical Outcome." *American Journal of Cancer Research* 6 (1): 61–70.

Galvao, Joana, Benjamin Davis, Mark Tilley,

Eduardo Normando, Michael R. Duchen, and M. Francesca Cordeiro. 2014. "Unexpected Low-Dose Toxicity of the Universal Solvent DMSO." *FASEB Journal* 28 (3): 1317–30. https://doi.org/10.1096/fj.13-235440.

Gersey, Zachary C., Gregor A. Rodriguez, Eric Barbarite, Anthony Sanchez, Winston M. Walters, Kelechi C. Ohaeto, Ricardo J. Komotar, and Regina M. Graham. 2017. "Curcumin Decreases Malignant Characteristics of Glioblastoma Stem Cells via Induction of Reactive Oxygen Species." *BMC Cancer* 17 (1): 1–11. https://doi.org/10.1186/s12885-017-3058-2.

Joven, Alberto, and András Simon. 2018. "Homeostatic and Regenerative Neurogenesis in Salamanders." *Progress in Neurobiology*, no. March: 0–1. https://doi.org/10.1016/j.pneurobio.2018.04.006.

Ma, Xingjun, Yisen Wang, Michael E Houle, Shuo Zhou, Sarah M Erfani, Sudanthi Wijewickrema, and James Bailey. 2018. "Ma18D.Pdf."

Mizukoshi, Kenji, Noriko Koyama, Toru Hayashi, Liguang Zheng, Sachiko Matsuura, and Masanori Kashimata. 2016. "Shh/Ptch and EGF/ErbB Cooperatively Regulate Branching Morphogenesis of Fetal Mouse Submandibular Glands." *Developmental Biology* 412 (2): 278–87. https://doi.org/10.1016/j.ydbio.2016.02.018.

Mohammad, Maeda H., Ahmed M. Al-Shammari, Ahmad Adnan Al-Juboory, and Nahi Y. Yaseen. 2016. "Characterization of Neural Stemness Status through the Neurogenesis Process for Bone Marrow Mesenchymal Stem Cells." *Stem Cells and Cloning: Advances and Applications* 9: 1–15. https://doi.org/10.2147/SCCAA.S94545.

Novak, Daniel, Laura Hüser, Jonathan J. Elton, Viktor Umansky, Peter Altevogt, and Jochen Utikal. 2020. "SOX2 in Development and Cancer Biology." *Seminars in Cancer Biology* 67 (August 2019): 74–82. https://doi.org/10.1016/j.semcancer.2019.08.007.

Oguchi, Ai, Chihiro Iwamoto, and Masashi Higuchi. 2024. "Differentiation Potential of SOX2-Positive Stem Cells in the Bovine Pituitary Gland." *Biochemical and Biophysical Research Communications* 738 (August): 150537. https://doi.org/10.1016/j.bbrc.2024.150537.

Singh, Dharmender, Vipin Kumar, and Virender Singh. 2020. "Et3N/DMSO-Supported One-Pot Synthesis of Highly Fluorescent β-Carboline-Linked Benzothiophenones via Sulfur Insertion and Estimation of the Photophysical Properties." *Beilstein Journal of Organic Chemistry* 16: 1740–53. https://doi.org/10.3762/BJOC.16.146.

Udalamaththa, Vindya Lankika, Chanika Dilumi Jayasinghe, and Preethi Vidya Udagama. 2016. "Potential Role of Herbal Remedies in Stem Cell Therapy: Proliferation and Differentiation of Human Mesenchymal Stromal Cells." *Stem Cell Research and Therapy* 7 (1): 1–8. https://doi.org/10.1186/s13287-016-0366-4.

Wang, Hongyu, Xifan Mei, Yang Cao, Chang Liu, Ziming Zhao, Zhanpeng Guo, Yunlong Bi, et al. 2017. "HMGB1/Advanced Glycation End Products (RAGE) Does Not Aggravate Inflammation but Promote Endogenous Neural Stem Cells Differentiation in Spinal Cord Injury." *Scientific Reports* 7 (1): 1–12. https://doi.org/10.1038/s41598-017-10611-8.

Yasunaga, Mayu, Fumiko Kobayashi, Yu Sogo, Kazutoshi Murotomi, Motohiro Hirose, Yuki Hara, Masashi Yamazaki, and Atsuo Ito. 2022. "The Enhancing Effects of Heparin on the Biological Activity of FGF-2 in Heparin–FGF-2–calcium Phosphate Composite Layers." *Acta Biomaterialia* 148: 345–54. https://doi.org/10.1016/j.actbio.2022.06.013.