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Neuroprotective effect of South Sulawesi propolis on neurogenesis in primary cultures of rat cerebral cortical neurons

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ABSTRACT

BACKGROUND

Neurodegenerative diseases have a significant risk factor, namely aging, which is associated with increased neuronal dysfunction and death. Propolis has been widely used as medicine due to its various benefits. This research study investigated the effect of propolis from the stingless bee (*Tetragonula sapiens*) from South Sulawesi, Indonesia, on neurogenesis in primary cultures of embryonic cerebral cortex of Wistar rats at 17-18 days of gestation.

METHODS

This research was an experimental study involving 4 female pregnant Wistar rats, which were terminated and the cerebral cortex of the embryos collected and grown as primary cultures. The cultures were divided into 3 groups, i.e. control, vehicle, and propolis extract group. The research began with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to obtain the optimal dose among propolis doses of 0.5 µg/mL, 1 µg/mL, 5 µg/mL, 10 µg/mL, 25 µg/mL, and 100 µg/mL. The study was continued by using the best dose in immunostaining examination using microtubule-associated protein 2 (MAP2) primary antibody and qRT-PCR examination of brain-derived neurotrophic factor (BDNF) mRNA expression. One Way ANOVA and Kruskal-Wallis test were used to analyse the data.

RESULTS

The results showed that the propolis doses of 0.5 µg/mL and 1 µg/mL significantly increase cell viability compared to the other doses ($p=0.011$) and stimulate dendritic growth. The propolis dose group of 1 µg/mL induces a significantly higher expression of BDNF mRNA than the control group ($p=0.031$).

CONCLUSION

Our findings indicate that stingless bee propolis has neuroprotective effects against BDNF mRNA in rats. It is shown that propolis can be a candidate inhibitor in neurodegenerative diseases.

Keywords: Propolis, neurodegenerative disease, brain-derived neurotrophic factor, rat

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INTRODUCTION

The global prevalence of elderly is rising, affecting an increasing number of degenerative diseases such as neurodegenerative diseases. Neurodegenerative disease is defined as a condition of progressive neuronal loss in the central nervous system (CNS), that may cause physical disability, cognitive deficits, or both. Alzheimer's disease and Parkinson's disease are the most common neurodegenerative diseases, characterized by behavioral disturbances and progressive cognitive dysfunction.⁽¹⁾ There are an estimated 6.2 million Americans above 65 years of age living with Alzheimer's, and this could potentially grow up to 13.8 million by 2060 if there are no medical developments to prevent, slow down or cure Alzheimer's disease.⁽²⁾

Neurodegeneration is a state where there is a degradation of the cell's ability to respond to stress, resulting in the disruption of cell homeostasis and protein formation.⁽³⁾ The neurodegenerative diseases are correlated with toxic protein deposition, such as in Alzheimer's disease, where the accumulation of amyloid- β peptides in the human brain triggers the dysfunction of synapses and neural networks that may cause cognitive deficits.⁽⁴⁾

The formation mechanism of neurodegenerative diseases has been targeted as a therapy and prevention, and several herbal medicines have been investigated that protect cells against free radicals and have antioxidant potential. One of these herbals is propolis, that is collected by honeybees from plants and has a number of important biological functions, including antitumor, immunomodulatory, anti-inflammatory, antioxidant, anticarcinogenic, antiviral, antimicrobial, antiparasitic and antidiabetic properties.⁽⁵⁾ The organic compounds that have been identified in propolis are polyphenols, terpenes, esters, amino acids, vitamins, minerals, and sugars.⁽⁶⁾ The other common constituents of propolis are organic acids, ketones, aldehydes and hydrocarbons.⁽⁷⁾ The bioactive molecular profile of raw propolis is variable depending on

geographical and botanical origin, season, bee genetics and environmental factors.⁽⁸⁾

Propolis contains chrysin, caffeic acid phenethyl ester (CAPE), and caffeoylquinic acid (CQA) that have neuroprotective effects.⁽⁹⁾ Previous studies proved that green propolis from Brazil can inhibit apoptosis in SH-SY5Y neuronal cell culture (a human neuroblastoma cell) exposed to staurosporine by inhibiting caspase-3 and apoptosis through an intrinsic pathway.⁽¹⁰⁾ Propolis extract reduced lactate dehydrogenase (LDH) production in cell line cultures of pheochromocytoma PC12 due to H₂O₂ exposure, thereby showing that propolis extract has a protective effect on mitochondria and neuronal cell membranes.⁽⁹⁾

In the neurological and neurodegenerative disease models, namely Alzheimer's disease, Parkinson's disease, and epilepsy, propolis also showed potential therapeutic benefits. Propolis was demonstrated to reduce amyloid fibrillation and reduce the impact of amyloid accumulation.^(10,11) Several phenol compounds in propolis can increase the expression of neuronal differentiation signalers (GAP-43, neurofilament light subunit, synaptophysin, synapsin) that are required for neuronal growth. Propolis may boost synapse formation through brain derived neurotrophic factor (BDNF),⁽¹²⁾ which is a neurotrophin that plays a leading role in the development, maintenance, repair and survival of neuronal populations. Some evidence shows that decreased function of neurotrophins and their receptors may cause neuronal injury and contribute to neurodegenerative diseases. Neurotrophins and bioactive compounds with the capability to activate neurotrophic receptors have a great potential in managing neurodegenerative diseases and other neurological disorders.⁽¹³⁾

There are various types of propolis growing in Indonesia and previous research examined the propolis content of stingless bees (*Tetragonula sapiens*) collected in South Sulawesi. This propolis has an antioxidant component from phenol compounds⁽¹⁴⁾ and is observed to be an effective antioxidant and anti-inflammatory. This

propolis has been studied based on the isolation conducted by Miyata et al.⁽¹⁵⁾ of propolis from *Tetragonula sapiens* from South Sulawesi, that contained the following flavonoid compounds: 2',3'-dihydro-3'-hydroxypapuanic acid; (-)-papuanic acid, (-)-isocalolongic acid, isopapuanic acid, isocalopolyanic acid, glyasperin A, brousoflavo Zero F; (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone, and isorhamnetin.

However, there is no study regarding the effect of propolis from South Sulawesi on neurogenesis of rat cortex primary cultures in the basal condition. Therefore this study aims to determine the effect of propolis on neurogenesis, by looking at its effect on cell viability, on BDNF mRNA expression, and on dendritic growth in rats.

METHODS

Research design

An in vitro experimental study was conducted at the Jakarta Litbangkes Animal Laboratory, the IMERI-UI Molecular Biology and Proteomics Core Facilities (MBPCF) Laboratory, and the Stem Cell and Tissue Engineering (SCTE) Laboratory from May 2022-November 2022.

Experimental animals

Female Wistar rats were obtained from the Animal Laboratory of Litbangkes Jakarta, with a pregnancy of 17-18 days, the initial weight of the rats before pregnancy being 250 grams. The animals were kept on a light/dark cycle for twelve hours on each cycle, at a temperature of $\pm 22^{\circ}\text{C}$, while feed and drinking water were served ad libitum. Accordingly, the total number of samples in this study was 4 female rats with 2 male rats to fertilize the females. Two female rats were used for MTS examination and the other two female rats were used for immunostaining and qRT-PCR of BDNF mRNA. Each group treatment was repeated in triplicate. All experiments were performed in accordance with the standard guidelines for the care and use of laboratory animals.

Reagents

Ketamine HCL (PT. Guardian Phartama Indonesia), Ilium Zylazil-100 (TROY LABORATORIES PTY. LIMITED), poly-D Lysine (Gibco), Corning® Laminin Surface (Merck), Dimethyl sulfoxide (Biomatik), D-(+)-glucose solution 45% (Gibco), Dulbecco's phosphate buffered saline (DPBS) modified 10x (Gibco), Neurobasal-A medium (Gibco), B-27 Supplement serum free (Gibco), L-glutamine 100x (Gibco), Papain Lyophilized (Sigma), bovine deoxyribonuclease I Type II (Sigma), Propolis Powdered Extract (CV. Nano Biotek Indonesia), Fetal Bovine Serum (Sigma), CellTiter 96 Aqueous Solution Cell Proliferation Assay (Promega), Paraformaldehyde 37% (Merck), PIPES (Sigma), Triton X-100 Surfact-Amps Detergent (Thermo Scientific), MAP2 Antibody (GeneTex), Vectafluor Excel Amplified Anti-Mouse IgG (Vector Laboratories), DyLight 488 Antibody Kit (Vector Laboratories), Vectashield Antifade Mounting Medium with DAPI (Vector Laboratories), QuickRNA Miniprep Plus isolation Kit (Zymo Research/California), synthesis kit for cDNA ReverTra Ace® qPCR RT Master Mix with gDNA remover (Toyobo/Osaka, Japan), real time PCR kit SensiFAST SYBR® Hi-ROX (Bioline/California), BDNF mRNA primer and housekeeping gene GAPDH (Integrated DNA Technologies).

Preparation of propolis extract

The propolis preparation used in this study was a product available on the free market that contained propolis extract powder. The extract doses used for the treatment were 0.5 $\mu\text{g}/\text{mL}$, 1 $\mu\text{g}/\text{mL}$, 5 $\mu\text{g}/\text{mL}$, 10 $\mu\text{g}/\text{mL}$, 25 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$. Each dose was dissolved in 1% DMSO. The primary culture was divided into 8 groups, namely medium (neurobasal growth medium) group as positive control, vehicle group, (neurobasal growth medium and 1% DMSO) and the groups given propolis extract at doses of 0.5 $\mu\text{g}/\text{mL}$, 1 $\mu\text{g}/\text{mL}$, 5 $\mu\text{g}/\text{mL}$, 10 $\mu\text{g}/\text{mL}$, 25 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$. The best dose in the MTS assay

was followed by immunostaining examination and measurement of BDNF expression, which was then compared with the control and vehicle groups. The MTS assay was carried out 24 hours after treatment, while the immunostaining examination and measurement of BDNF expression were carried out on the 7th day after treatment. Each treatment group was tested in triplicate.

Neuronal cell culture

Pregnant female Wistar rats at 17-18 days gestation were terminated with 90mg/kg ketamine and 10mg/kg xylazine. The abdomen was cleaned with 70% ethanol and an incision made to open the peritoneal space. After the amniotic sac was located, the embryo was removed from the uterus and transferred to a petri dish filled with phosphate-buffered saline (PBS). We used PBS 1x made into 1L and autoclaved at 121°C for 20 minutes. One day before rat dissection, incubation was performed by using poly D-lysine substrate at 0.1 mg/mL in 12 wells that had been placed on a 1mL cover slip of 18 mm diameter (until submerged). Incubation was conducted at 4°C overnight. On the next day, the poly D-lysine was aspirated and the well washed three times with PBS. The well was incubated with 600 µL/well of Laminin at 5 µg/mL concentration for 4 hours at 4°C. After the rat termination process, the rat embryo cortex isolation process was performed; the rat skull was fixed at the back and the lower part of the head was trimmed. After the brain was released from the meninges, the clean cortex was taken from the brain. The clean cortex was transferred to a 15 mL phial filled with PBS glucose. After the microdissection was completed, the PBS glucose was discarded and papain solution (10 mg papain in 1 ml sterile distilled water) was added and incubated for 15 minutes at 37°C. For papain inactivation, PBS/glucose, B27 supplement, and DNase was added and incubated for 5 minutes at 37°C. The dissociation and centrifugation were conducted at 1000 rpm for 5-10 minutes. The supernatant was discarded and BSA cushion was added (BSA and neurobasal growth medium that

had been filtered and homogenized). Then re-centrifugation was done at 120 g for 6 minutes and the supernatant was discarded and resuspended with 5 mL of neurobasal growth medium. Neurobasal growth medium consists of 48.7 mL neurobasal supplemented medium, 50 µL lactic acid, 1 mL B27 supplement, 250 µL L-glutamine, 1% amphotericin B and 1% penicillin-streptomycin. Cells were grown in each 12-well plate at 300,000 cells per well and in 96-well plates at 10,000 cells per well. Before the cells were grown in the wells, the laminin was aspirated before incubation for 4 hours and the wells washed three times with PBS.

Cell viability assay

The 96-well plates were prepared in triplicate at 10,000 cells/well and divided into 8 groups, consisting of medium as the control group, vehicle group, and propolis extract groups at doses of 0.5 µg/mL (P05), 1 µg/mL (P1), 5 µg/mL (P5), 10 µg/mL (P10), 25 µg/mL (P25) and 100 µg/mL (P100). After being soaked for 24 hours and observed for any changes in cell culture, 100 µl of culture medium was added to the MTS solution (10 µl MTS stock solution 5 mg/ml). They were incubated for 4 hours at 37°C in an incubator with 5% CO₂. Cells were observed after 4 hours to examine the formation of formazan salts and the absorbance at 490 nm was recorded.

Observations of morphological changes

Cells were observed until day 7 post treatment which consists of medium, vehicle and the two best doses, namely 0.5 µg/mL and 1 µg/mL, were selected after the MTS assay procedure. Cellular morphology was observed and captured for each day by using an inverted microscope (Nikon Eclipse Ti-S).

Immunohistochemistry

On day in vitro (DIV) 7, coverslips in wells that consisted of control, vehicle, and best dose propolis groups were subjected to immunostaining procedures. The medium was aspirated from the

culture disk and was washed 3 times with PBS 1x. The culture disk was fixed with 4% paraformaldehyde, incubated for 15 minutes, then washed 3 times with PBS 1x and permeabilized with 0.1% Triton-100 for 10 minutes, followed by 3 washings with PBS 1x. Afterwards it was incubated with 2-5% normal horse serum for 20 minutes and washed 3 times with PBS 1x. The culture disk was incubated with polymer antibody (MAP 2) at 1:250 overnight at 4°C and washed 3 times with PBS 1x. The culture disk was incubated with antibody amplifier for 15 minutes and with VectaFluor Amplified Dylight 488 anti-rabbit IgG for 30 minutes, followed by washing 3 times with PBS 1x after each incubation was completed. For preserving fluorescence the slide was mounted with Vectashield Antifade Mounting Medium with DAPI. The slides were stored in the dark, protected from light at 4°C until imaging. Observations were made with a 20x objective on a confocal microscope (Zeiss LSM 700).

Real-time quantitative polymerase chain reaction (qRT-PCR)

mRNA was isolated from cultured cells of rat embryonic cortical neurons. Extracted RNA at a concentration of 5 µg/mL was reverse transcribed to cDNA using ReverTra Ace® qPCR RT Master Mix with gDNA remover cDNA synthesis kit (Toyobo/Osaka, Japan). After polymerase activation was performed at 95°C for 2 minutes, it was followed by 95°C denaturation for 5 seconds with 40 cycles and 60°C annealing for 30 seconds. cDNA was amplified by a real time PCR instrument (Applied Biosystem) with the SensiFAST SYBR® Hi-ROX real time PCR kit (Bioline/California). The primer pair sequences were as follows: BDNF mRNA primer 5'-TACGAGACCAAGTGCAATCC-3' (forward); 5'-TCGCCAGCCAATTCTTT-3' (reverse) and GAPDH primer 5'-ACCACAGTCCATGCCATCAC-3' (forward); 5'-TCCACCACCCTGTTGCTGTA-3' (reverse). GAPDH primer was used as a housekeeping gene. Relative expression was calculated by Livak's formula and each treatment group was processed in triplicate.

Statistical analysis

Data were presented as mean ± standard error of the mean. Statistical analysis was completed by One Way ANOVA with LSD post hoc test as parametric test and Kruskal-Wallis test followed by multiple comparisons between the groups as nonparametric test using SPSS (IBM SPSS Statistic). GraphPad Prism (Dotmatics) software was used for making graph. A $p < 0.05$ score was considered to indicate statistical significance.

Ethical clearance

All procedures involving animals were conducted in line with the research protocol approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Indonesia, and Dr. Cipto Mangunkusumo National General Hospital, under No. KET-518/UN2.F1/ETIK/PPM.00.02/2022 and protocol No. 22-04-0446 on May 30, 2022

RESULTS

Effect of propolis extract on cell viability

Cell viability in this study was measured by using MTS, a calorimetric method to assess cell metabolic activity. The MTS assay is applied to assess cell proliferation, cell viability and cytotoxicity. **Figure 1** shows an overview of the primary culture of rat cerebral cortex neurons

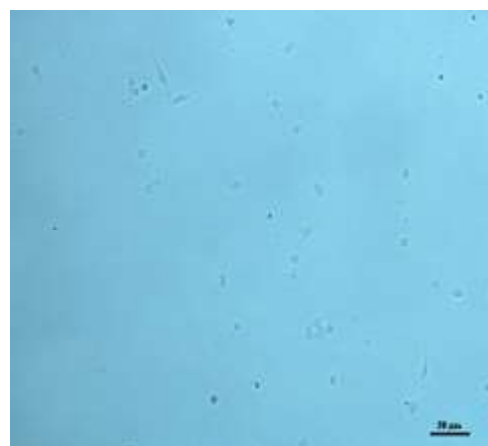


Figure 1. The overview of neuronal cells before MTS treatment contains immature neurons

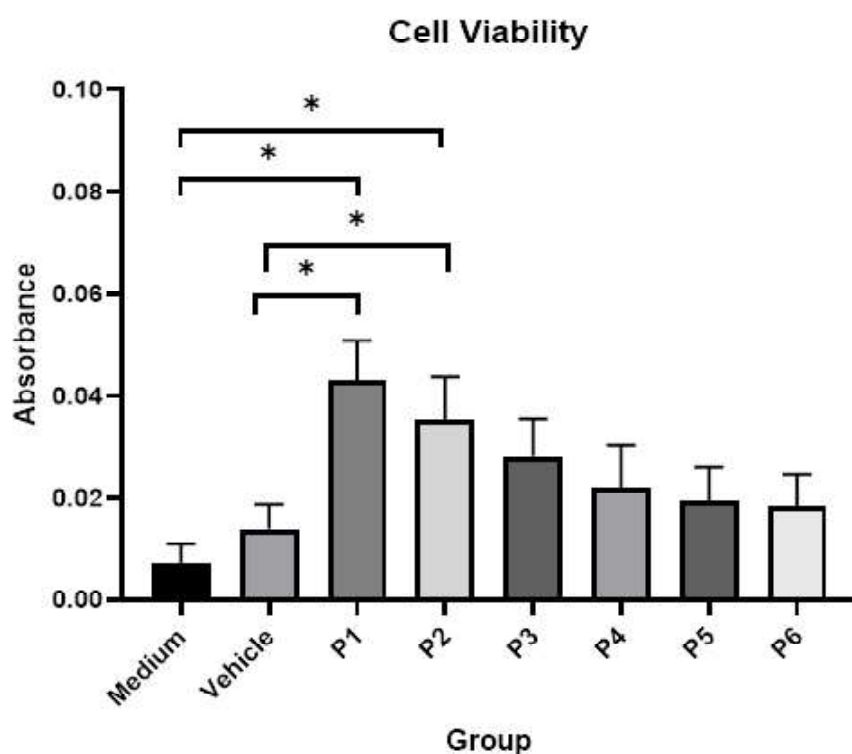


Figure 2. The absorbance of cell viability on the first day after treatment

Data presented as mean \pm SEM. One way ANOVA with LSD post hoc analysis. $n=3$ rats/group. (Medium: normal control; Vehicle: medium with 1% DMSO solvent; P1: 0.5 μ g/mL propolis; P2: 1 μ g/mL propolis; P3: 5 μ g/mL propolis; P4: 10 μ g/mL propolis; P5: 25 μ g/mL propolis; P6: 100 μ g/mL propolis).

before the MTS solution was given, while the treatment was conducted on day 1 of primary culture. This parameter was assessed on the day after treatment for each group (Figure 2).

Data analysis used one-way ANOVA; the results showed there were differences between groups ($p=0.011$). From these results, there were two highest absorption values in the dose of propolis given 0.5 μ g/mL and 1 μ g/mL compared to the other groups. The P0.5 group was significantly different from the medium ($p=0.005$), vehicle ($p=0.032$), P100 ($p=0.013$), P25 ($p=0.018$) and P10 ($p=0.032$) groups. On the other hand, the P1 group was significantly different from the medium ($p=0.005$) and vehicle ($p=0.032$) groups. Thus, the 0.5 μ g/mL and 1 μ g/mL doses of propolis were found to result in better cell viability compared to the medium and vehicle ground. These two doses were then used as the selected doses for tests on the other parameters, such as immunostaining and BDNF mRNA expression test.

Effect of propolis extract on qualitative analysis of dendrite expression through MAP2 primary antibody

Through the MTS test, two selected doses were obtained and used for the immunostaining test. The primary cell culture results of the rat cerebral cortical neurons were observed until the seventh day after treatment.

Figures 3, 4, 5, and 6 give a description of the growth in primary cultures of rat cerebral cortical neurons on day 1 after treatment until day 7 after treatment. The results show neuronal cell growth in all groups, such as medium, vehicle, propolis 0.5 μ g/mL, and propolis 1 μ g/mL groups. The yellow arrowhead in the figure points to a neuronal dendrite. On DIV 1-3 the dendrite is still short and immature, and in DIV 4-7 the dendrite continues to grow, thicken and branch. After 7 days post-treatment, the cells were fixed and treated for immunostaining with MAP2 primary antibody to see the image of dendrites in the cells. **Figure 7** shows the results of

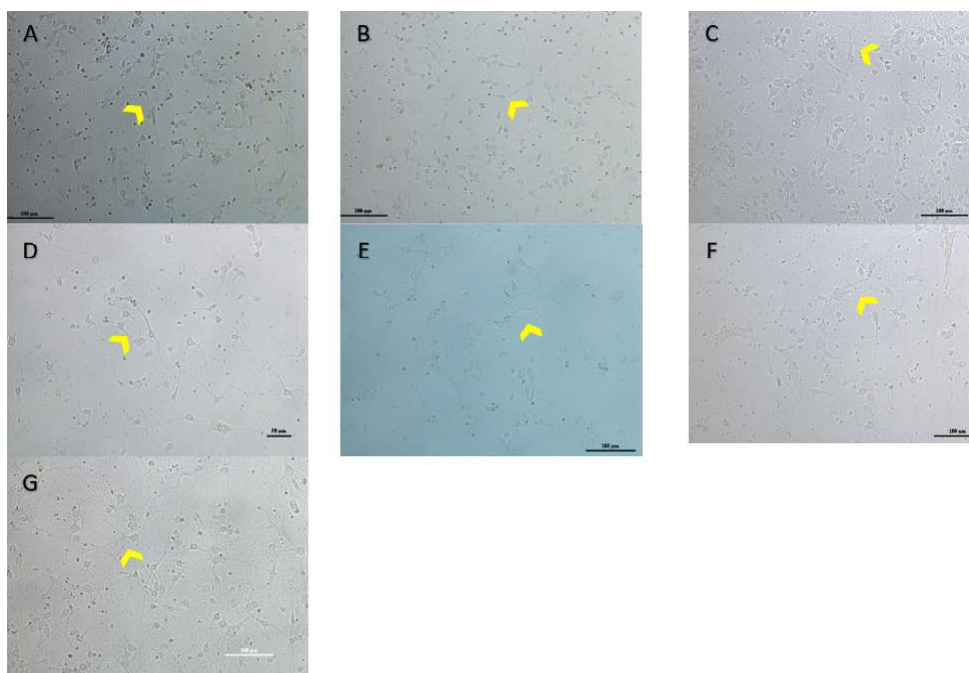


Figure 3. The effect of the propolis extracts on rat cerebral cortex neuron cells after the intervention with medium. Arrows indicate the dendrite growth. Group (A) Day 1 after intervention (B) Day 2 after intervention (C) Day 3 after intervention (D) Day 4 after intervention (E) Day 5 after intervention (F) Day 6 after intervention (G) Day 7 after intervention. On days 1-3 after intervention the dendrites are still short and immature, and on days 4-7 after intervention the dendrites continue to grow, thicken and branch out.

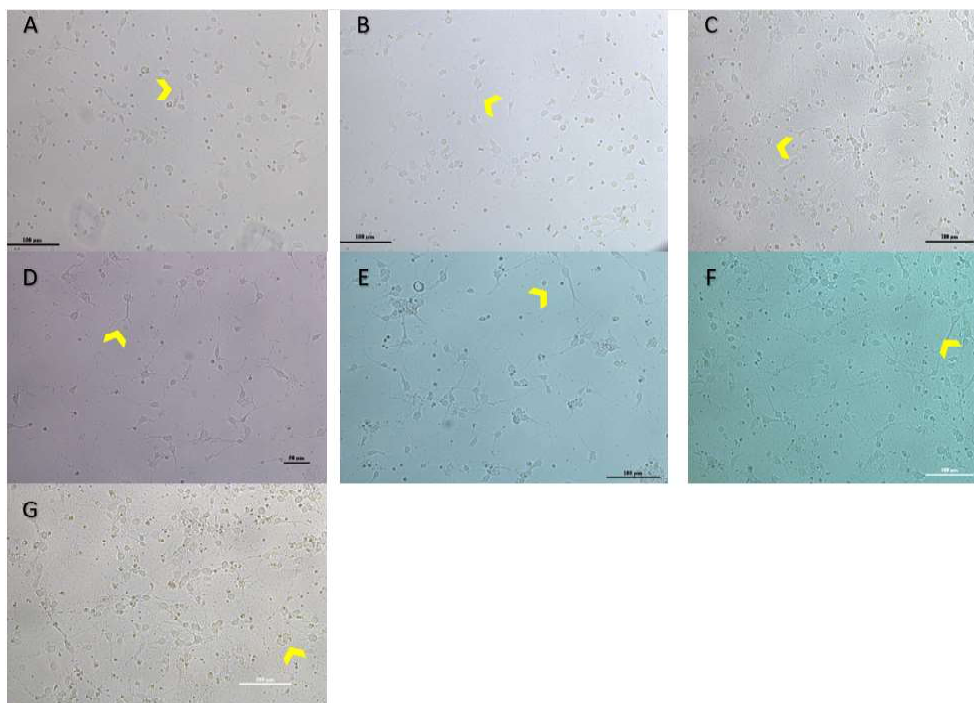


Figure 4. The effect of the propolis extracts on rat cerebral cortex neuron cells after the intervention with vehicle. Arrows indicate the dendrite growth. Group (A) Day 1 after intervention (B) Day 2 after intervention (C) Day 3 after intervention (D) Day 4 after intervention (E) Day 5 after intervention (F) Day 6 after intervention (G) Day 7 after intervention. On days 1-3 after intervention the dendrites are still short and immature, and on days 4-7 after intervention the dendrites continue to grow, thicken and branch out.

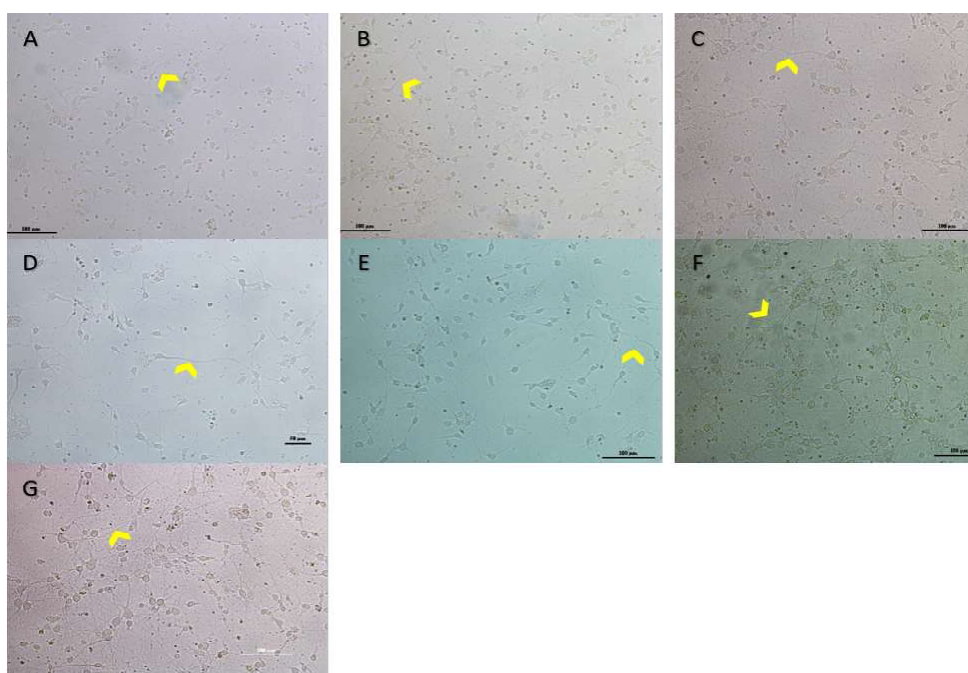


Figure 6. The effect of the propolis extracts on rat cerebral cortex neuron cells after the intervention with $1\mu\text{g}/\text{mL}$ dose of propolis. Arrows indicate the dendrite growth. Group (A) Day 1 after intervention (B) Day 2 after intervention (C) Day 3 after intervention (D) Day 4 after intervention (E) Day 5 after intervention (F) Day 6 after intervention (G) Day 7 after intervention. On days 1-3 after intervention the dendrites are still short and immature, and on days 4-7 after intervention the dendrites continue to grow, thicken and branch out

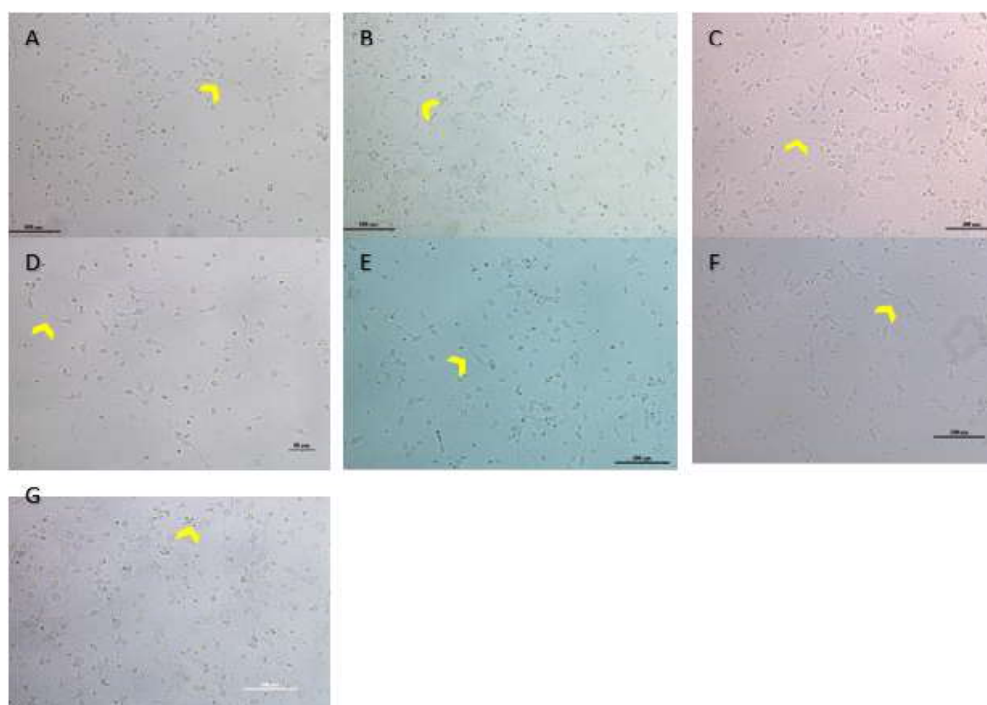


Figure 5. The effect of the propolis extracts on rat cerebral cortex neuron cells after the intervention with $0.5\mu\text{g}/\text{mL}$ dose of. Arrows indicate the dendrite growth. Group (A) Day 1 after intervention (B) Day 2 after intervention (C) Day 3 after intervention (D) Day 4 after intervention (E) Day 5 after intervention (F) Day 6 after intervention (G) Day 7 after intervention. On days 1-3 after intervention the dendrites are still short and immature, and on days 4-7 after intervention the dendrites continue to grow, thicken and branch out.

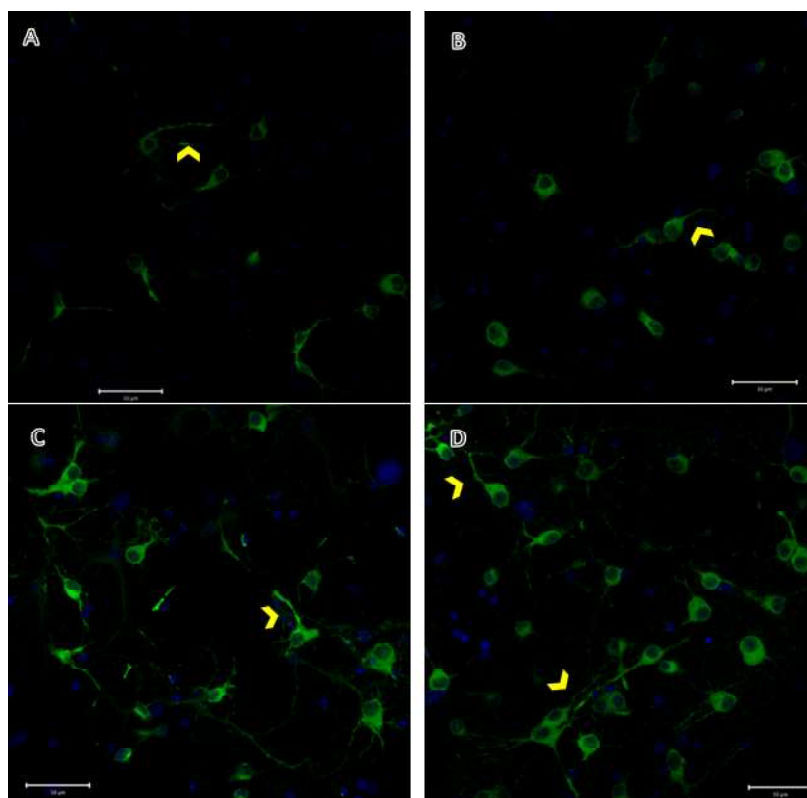


Figure 7. Overview of cerebral cortex neuronal cultures of day in vitro (DIV) 7 mouse embryos post-treatment utilizing MAP2 immunostaining technique. Arrows indicate the dendrite growth. Group (A) Medium and (B) Vehicle show fewer cells, shorter dendrites, compared to Group (C) 0.5 μ g/mL dose propolis and (D) 1 μ g/mL dose of propolis that has thicker and branched dendrites.

Relative BDNF mRNA Expression

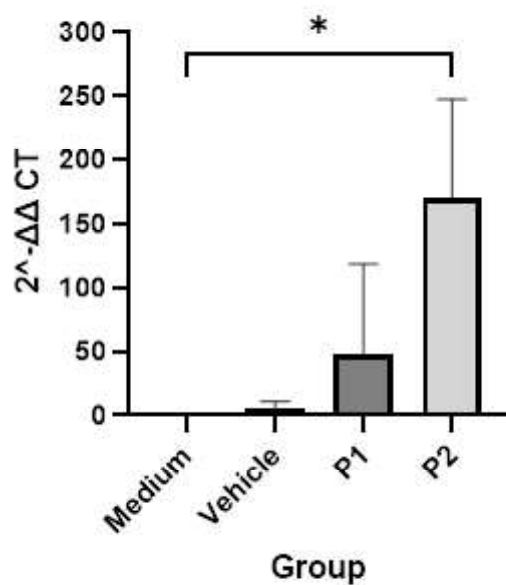


Figure 8. Brain-derived neurotrophic factor mRNA expression

Data presented as mean \pm SEM. Kruskal-Wallis test followed by multiple comparisons between the groups. n=2 rats/group. (Medium: normal control; Vehicle: medium with 1% DMSO solvent; P1: 0.5 μ g/mL propolis; P2: propolis 1 μ g/mL)

immunostaining with MAP2 primary antibody in each group.

Figure 7 shows that all groups express cell staining by using MAP2 primary antibody with different characteristics for each treatment, indicating that neuronal cells in the medium, vehicle, P0.5 and P1 groups express MAP2 protein as dendrite signaling in neuronal cells.

Effect of propolis extract on BDNF mRNA expression in rat cerebral cortex neuron cell cultures

The results of BDNF mRNA expression analysis in rat cerebral cortical neuron cultures tested by real time PCR are shown in Figure 8. The mean value of BDNF mRNA relative expression in the medium group was 1, in the vehicle group it was 5.972409, in group P0.5 47.08719 and in group P1 169.6081. According to the normality test, the BDNF mRNA relative expression data were not normally distributed and homogeneous, therefore the data were analyzed by using the Kruskal Wallis test. This study showed that there was a significant difference in the relative expression of BDNF mRNA in each group ($p=0.031$).

The data from **Figure 8** shows that there are significant differences between groups on the relative expression of BDNF mRNA, which in group P1 is significantly different ($p=0.018$) from the medium group. The 1 $\mu\text{g}/\text{mL}$ dose of propolis expressed higher BDNF mRNA than the medium group.

DISCUSSION

The results in this study showed that 0.5 $\mu\text{g}/\text{mL}$ and 1 $\mu\text{g}/\text{mL}$ doses of propolis showed significantly better results in MTS examination compared to the medium and vehicle. This implies that the 0.5 $\mu\text{g}/\text{mL}$ and 1 $\mu\text{g}/\text{mL}$ doses of propolis result in better cell viability compared to the medium and vehicle. Propolis contains various neuroprotective agents such as chrysin, caffeic acid phenethyl ester (CAPE) and caffeoylquinic acid (CQA) that may inhibit apoptotic cells

through the inhibition of caspase-3 and intrinsic apoptotic pathways. Caffeic acid phenethyl ester can reduce malondialdehyde (MDA) as a lipid peroxidation biomarker, therefore it can inhibit harmful agents that cause aging.^(5,16,17)

In the study by Ni et al.,⁽¹⁸⁾ the investigators examined the effect of Brazilian green propolis on cell viability in an in-vitro study using SH-SY5Y human neuroblastoma cells which were previously exposed to ROS. They found that the viability of cells given propolis was higher than that of cells not given propolis. This showed that propolis has a protective effect on neuronal cells. The propolis used in this study was from stingless bees (*Tetragonula sapiens*) and was collected in South Sulawesi, Indonesia. This propolis has been known to be rich in polyphenols that are high sources of antioxidants. South Sulawesi propolis has the potential as a COX-2 inhibitor that may inhibit inflammation, and has protective effects against oxidative stress.⁽¹⁹⁾

In this study, the optimal doses of propolis were 0.5 $\mu\text{g}/\text{mL}$ and 1 $\mu\text{g}/\text{mL}$, while there was a decrease in cell viability at higher doses. Flavonoids can prevent the toxic effects of active metals such as Fe or Cu, but in certain circumstances they can also act as pro-oxidants and increase the oxidation of molecules. The role of propolis as antioxidant or pro-oxidant depends on the dose used, therefore propolis doses above the optimal dose can exert pro-oxidant effects and cause cell damage.^(20,21)

Neurodegenerative diseases occur due to alterations in the brain, as a result of age-dependent changes that can be stimulated by neuroinflammation and ROS, causing neurodegeneration.⁽¹⁾ In this study, it was found that propolis from South Sulawesi has the effect of improving cell proliferation and has a protective effect on neuronal cells compared to the medium and vehicle. Thus, propolis can be used as a preventive agent against neurodegenerative diseases.

The immunostaining test with MAP2 primary antibody showed a positive luminescence, indicating the expression of MAP2 protein in the

neuronal cells. It showed the dendrite growth in the propolis group from South Sulawesi at doses of 0.5 µg/mL and 1 µg/mL, as compared to medium, and vehicle. The results showed that the groups receiving 0.5 µg/mL and 1 µg/mL doses of propolis had thicker, longer, and branched dendrites compared to the medium and vehicle groups.

The measurement of dendrite length in each group was not yet possible, because the images still do not meet the requirements for the calculation of dendrite values, such as the distance between neurons that is still too close, and there are discontinuous lines on the dendrites causing inaccurate calculations.

This study used MAP2 primary antibody, as an outgrowth signaling protein. Propolis contains CAPE which has been shown as an effective modulator of neurogenesis, dendrite formation, elongation and branching as well as inhibiting the deficiency that was induced by neurotoxins. A number of proteins for neurogenesis are stimulated by propolis, including growth-associated protein 43 (GAP-43), microtubule-associated protein (MAP), tau, synaptophysin, and synapsin.⁽¹²⁾

This study showed through qRT-PCR analysis that the group receiving South Sulawesi propolis at a dose of 1 µg/mL expressed BDNF mRNA significantly higher than did the medium group. In a study by Kuswati et al.⁽²²⁾ using rats that had previously experienced induced stress for 14 days, it was found that BDNF expression in the hippocampus was significantly higher in the group given propolis at a dose of 200 mg/kgBW compared to those not given propolis. The latter study is in line with our study which showed that BDNF mRNA expression in the group given propolis was higher than that in the medium group as a positive control.

Propolis is rich in polyphenolic compounds, such as CAPE, curcumin, and quercetin that are reported to elevate BDNF levels. BDNF activation by several polyphenolic compounds increases cell viability and antiapoptotic signaling.⁽¹²⁾ An increase in BDNF expression

was found in the research of Ni et al.⁽¹⁸⁾ on the cytotoxicity in SH-SY5Y human neuroblastoma cells induced by hydrogen peroxide, with the result that Brazilian green propolis significantly increased BDNF mRNA expression mediated by PI3K signaling.

The limitation of the study is that it cannot calculate dendrite length because there are discontinuous lines on the dendrites, so further optimization of immunostaining using MAP2 antibodies is needed. The clinical implication of these study results is that propolis may be used to inhibit or prevent neurodegeneration. Further studies are needed regarding the effect of propolis on pathological conditions because this study only examined the physiological condition.

CONCLUSION

This study demonstrated that utilized propolis from South Sulawesi can significantly increase cell viability. Propolis also expresses dendritic signalers through immunostaining with MAP 2 primary antibody. Meanwhile, in the test of BDNF mRNA expression, propolis proved to more effectively increase BDNF mRNA expression compared to the medium group.

CONFLICT OF INTERESTS

The authors declare no conflict of interests in association with this study.


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AUTHOR CONTRIBUTIONS

IRS contributed to writing the protocol and revised the manuscript. NI and TA revised the manuscript. AY contributed to the design of the study and wrote the first draft of the manuscript. All authors have read and approved the final manuscript. 

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