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EFFECT OF ETHANOL EXTRACT FROM KEPEL LEAVES (*Stelechocarpus Burahol* (Bl.) Hook F. & Th.) ON CONFLUENCE AND VIABILITY OF PRIMARY MOUSE (*Mus musculus*) NEURONAL CELLS

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Abstract

Kepel (Stelechocarpus Burahol (Bl.) Hook F. & Th.), a plant indigenous to Indonesia, is popular for its various benefits, including anti-hyperuricemia, xanthine oxidase inhibition, anti-implantation, anticancer, antiseptic, anti-inflammatory, and antioxidant properties. However, its potential as a neuroprotective agent is not well understood. Therefore, this study aims to investigate the confluence and viability of primary mouse neurons following the administration of ethanol extract from Kepel (Stelechocarpus burahol (Bl.) Hook F. & Th.) leaves. Primary neuron cell cultures were divided into four treatment groups: NT (without treatment) as a control group, T1 (50 µL of Kepel leaf extract), T2 (75 µL of Kepel leaf extract), and T3 (100 µL of Kepel leaf extract). After 48 hours of incubation, culture confluence and viability were assessed. This study approved that the ethanol extract of Kepel leaves did not significantly affect confluence compared to the control, but cell viability increased when treated with the Kepel leaf extract compared to the control.

1. INTRODUCTION

The Kepel plant (*Stelechocarpus burahol* (Bl.) Hook F. & Th.), native to Indonesia, has been traditionally used for gout relief and is popular for its natural fragrance properties. It is associated with various biological activities such as anti-hyperuricemia, anti-implantation, anticancer, anti-inflammatory, antiseptic **[1]**, xanthine oxidase inhibition **[2]**, antibacterial **[3]** and antioxidant effects **[4]**. Elfasyari and Marliza **[5]** highlight the prevalence of flavonoids in Kepel, with the highest concentrations found in ethanol extracts of mature leaves. Flavonoids are recognised for their potential neuroprotective benefits **[6, 7, 8]**.

Primary neuron cell cultures are important tools for studying both neurodegeneration and potential neuroprotective agents. Neurodegeneration is not solely linked to age-related cognitive conditions such as

Alzheimer's, Parkinson's, and Huntington's diseases, nor is it solely caused by neuronal decline. Multiple factors contribute to these diseases, including abnormal protein aggregation, synaptic and neuronal damage, disruptions in proteostasis, cytoskeletal abnormalities, shifts in energy balance, DNA and RNA defects, inflammation, and neuronal cell death **[9; 10]**.

Despite the limited research on the neuroprotective potential of Kepel, this study aims to investigate the effectiveness of Kepel leaf extract as a neuroprotective agent in vitro by examining the confluence and viability of primary mouse neurons following the administration of ethanol extract from Kepel (*Stelechocarpus burahol* (Bl.) Hook F. & Th.) leaves.

2. MATERIALS AND METHODS

Materials

The leaves of Kepel collected from Cageran Village, Yogyakarta, Indonesia.

Exstraction of Kepel Leaves

The extraction procedure involves the maceration method, where 100 grams of Kepel leaf powder is soaked in 500 ml of 96% ethanol (at a ratio of 1:5) for two 24-hour periods. After these two soaking cycles, the mixture is filtered through filter paper. This is followed by a second maceration step where another 500 mL of 96% ethanol (at the same ratio) is added, and the mixture undergoes two additional 24-hour cycles of soaking. The soaked mixture is then filtered again using filter paper, and the resulting residue is evaporated using a Rotary Evaporator. Once the extraction process is completed, the resulting extract is obtained in paste form and is ready for use.

Preparation of Culture Media (DMEM Stock)

To prepare the culture media, precise amounts of 1.35 grams of DMEM, 0.37 grams of $NaHCO_3$, 0.006 grams of Penicillin, 0.01 grams of Streptomycin, and 0.23 grams of Hepes were accurately weighed. These components were dissolved in 100 mL of distilled water. The resulting mixture was then filtered using a 0.22 µm Millipore membrane, and the prepared culture media stock was subsequently stored at 4°C.

Isolation and Cell Culture of Mouse Neurons (*Mus musculus*)

The mouse (*Mus musculus*) used were aged between 3 to 5 days. After euthanasia, their heads were dissected to extract the brains. The brain tissues were carefully rinsed with 2 mL of NaCl solution to remove debris. The tissues were then minced and suspended in NaCl solution using a syringe. The suspended tissues were transferred into 1 mL of media in 15 µL tubes. These tubes were centrifuged at 2000 rpm for 5 minutes, after which the supernatant was removed and the pellet was resuspended in 2 mL of DMEM culture medium. The suspension underwent another centrifugation step at 2000 rpm for 5 minutes. The resulting pellet was transferred to a 90 mm petri dish for cell culture and homogenized by agitation. Finally, the dish was placed in an incubator set at 37°C with 5% CO_2 for 48 hours.

Maintenance the Neuronal Cell

Primary neuron cell cultures were divided into four treatment groups: NT (without treatment), T1 (50 µL of Kepel leaf extract), T2 (75 µL of Kepel leaf extract), and T3 (100 µL of Kepel leaf extract).

Observation of Mouse Neuron Cell Confluence

Cell confluence assessments were performed to evaluate the growth and density of neuronal cells under different treatments. The extent of neuronal cell adherence to the substrate and their spread was observed using an inverted microscope and analyzed using imaging software.

Observation of Mouse Neuron Cell Viability

Neuronal cell viability is assessed to determine the proportion of living and healthy cells. Cultured cells are harvested by removing the medium, washing with PBS, and then treating with 1 ml of trypsin to detach them. After incubating for 5 minutes to achieve single cells, the cells are centrifuged with 1 ml of PBS added. A 25 µl cell suspension is taken, and then 10 µl of cells are mixed with 10 µl of trypan blue and thoroughly blended. A 10 µl portion of the cell suspension containing trypan blue is placed on a disposable glass slide for counting using an automatic cell counter (Invitrogen Thermo Fisher).

Data Analysis

The ANOVA test was used to determine the differences in viability and confluence of Mus musculus neuronal cells in vitro following treatment with ethanol extracts of Kepel leaves. Data with $p<0.05$ was assumed to represent statistical significance.

3. RESULTS

The Confluence of Neuronal Primary Cell

Confluence was measured 48 hours after incubation to assess the density and growth of the neuron cells. It is means value of confluence indicates successful cell proliferation and expansion.

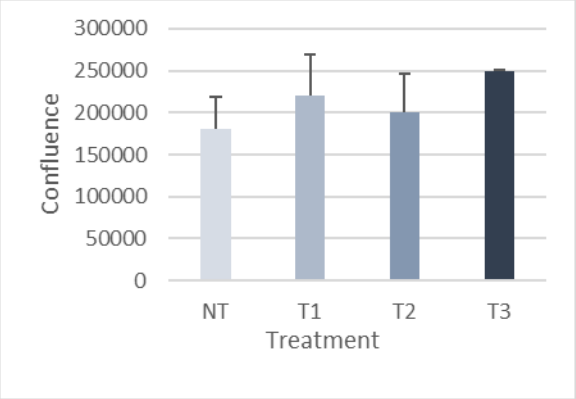

Figure 1. The confluence of neuronal primary cell culture. (NT: Non treated, normal cell; T1: treated with 50 µL of Kepel leaf extract; T2: treated with 75 µL of Kepel leaf extract; and T3: treated with 100 µL of Kepel leaf extract)

The result shows that confluence on both non-treated and treated with ethanol extracts of Kepel not significantly different. However, confluence in treated groups (T1, T2, T3) higher than non-treated group (NT) (Figure 1).

The Viability of Neuronal Primary Cell

The viability of neuronal primary cell was counted on 48 hours after incubation. It is performed to know the proportion of living cells.

In Figure 2 show that viability of neuronal primary cell was significantly different between non-treated group and treated group. The treated group has higher viability than non-treated group. Furthermore, T1 as treated with 50 µL of Kepel leaf extract has highest viability value.

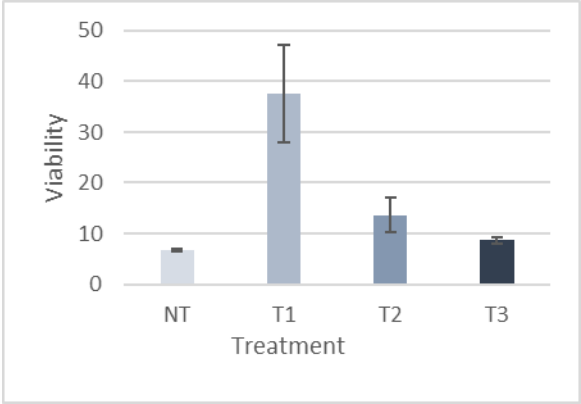

Figure 2. The viability of neuronal primary cell culture. (NT: Non treated, normal cell; T1: treated with 50 µL of Kepel leaf extract; T2: treated with 75 µL of Kepel leaf extract; and T3: treated with 100 µL of Kepel leaf extract).

4. DISCUSSION

Although confluence and viability are related, they assess different aspects of cell culture. Confluence evaluates cell growth and density, while viability measures cell health and functionality. These two parameters are very important to determine cell health and optimal conditions of experimental.

This study indicated that there was no significant difference in confluence between the non-treated (NT) and the treated of ethanol extract of Kepel leaves (T1, T2, T3). This suggests that the ethanol extract of Kepel leaves is non-toxic and does not reduce the culture's confluence. Additionally, it shows that the proliferation of primary neuron cells is not affected by the ethanol extract content of Kepel leaves.

Kepel leaves contain flavonoid as Ramadhan et al. **[11]** reported that mature Kepel leaves possess the highest flavonoid content compared to medium-aged and young leaves. According to Elfasyari and Marliza **[5]** the flavonoids present in mature Kepel leaves are primarily flavonones and flavones. The result suggested that might be flavonoid type in Kepel leaves not influence the cell proliferation.

However, different results were observed in the viability parameters, where the viability of neuron cells treated with ethanol extract of Kepel leaves was higher than non-treated (NT). This indicates that the active compounds in Kepel leaves can increase the proportion of living cells in primary neuron cell cultures. The high viability indicates that most cells are still alive and metabolically active. This aligns with Vauzour et al. **[7]**, who stated that flavonoids influence neurogenesis through neuronal signaling pathways in the brain, preventing apoptosis caused by neurotoxic agents and fostering neuronal survival and differentiation. Furthermore, flavonoids have positive effects on the peripheral and cerebral vascular systems, enhancing cerebrovascular blood flow. Dajas et al. **[6]** stated that flavonoids can protect the brain by modulating intracellular signals that promote cell survival. Moreover, flavonoid perform a neuroprotective function by depressing neuroinflammation **[8]**.

5. Conclusion

In this study, the finding suggests that while the ethanol extract of Kepel leaves may enhance cell viability, it does not significantly impact confluence. However, the lack of effect on confluence does not necessarily mean the components in the extract are less beneficial.

6. ACKNOWLEDGEMENTS

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