Coffea canephora Bean Extract Induces NIH3T3 Cell Migration

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BACKGROUND: Wound healing is an essential biological process that consists of sequential steps aimed at restoring the architecture and function of damaged cells and tissues. There are empirical evidences of using pure coffee bean powder as an alternative medicine in treating various types of wounds. However, there is limited data on coffee-induced wound healing, especially migration of cells. Therefore, current study was conducted to investigate the role of coffee extract in cell migration, especially fibroblast which is important for wound healing.

METHODS: Coffea canephora beans were prepared, extracted and added in the NIH3T3 cell culture in final concentration of 2.5% and 5%. Then cytotoxicity test was performed using Na30-[{(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzenesulfonic acid hydrate (XTT) assay. Meanwhile, cell migration test was performed with scratch assay. All results were statistically analyzed.

RESULTS: The 2.5% or 5% Coffea canephora beans extract (CCBE)-treated NIH3T3 cell numbers were almost similar with the numbers of NIH3T3 cells in starvation medium merely. Meanwhile, 2.5% and 5% CCBE showed significant decrease of the widths of scratched areas compared to starvation medium merely (ANOVA with LSD Post-hoc, p=0.000). After 24 h and 48 h, the average widths of 2.5% and 5% CCBE-treated scratched areas were 235.68±22.79, 50.36±5.29, 229.95±23.01, 27.68±2.83, respectively.

CONCLUSION: Since both 2.5% and 5% CCBE are potential in inducing migration of fibroblast (NIH3T3 cell) and do not induce cytotoxicity, the CCBE could be potential as an agent for wound healing.

KEYWORDS: coffee, Coffea canephora, NIH3T3, migration, cytotoxicity

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preferred for treatments.(5) There are empirical evidences that Indonesians have long been using pure coffee bean powder as an alternative medicine in treating various types of wounds. A series of studies on coffee bean powder since the beginning of 2004 has shown that it can be used to treat various types of wounds, ranging from sharp scratches, burns to infected wounds.(6) In animal model, infected wound healing using coffee bean powder was shown to be superior than other traditional agents, such as honey.(7) Coffee bean powder has many useful ingredients, including alkaloids, saponins, flavonoids, phenolic compounds and chlorogenic acid.(8)

From several previous studies, it was estimated that flavonoids as anti-inflammatory, while saponins as antibiotics to inhibit bacterial growth in order to accelerate wound healing.(9) Coffee bean powder can be a potential agent for wound healing, and it is possible that there is a coffee ingredient that can induce the cell migration. However, there is limited data on coffee extract/ingredient-induced migration of cells. Therefore, current study was conducted to investigate the role of coffee extract/ingredient in cell migration, especially fibroblast which is important for wound healing.

Methods

Coffea canephora Bean Extract (CCBE) Preparation
Coffee (C. canephora) beans were cleaned, blended, mixed with 80% ethanol for 3 hours, macerated for 24 hours, filtered and evaporated. Resulted CCBE paste was diluted in starvation medium containing Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) 2% Fetal Bovine Serum (FBS) (Sigma-Aldrich) and 1% Penicillin-Streptomycin (Sigma-Aldrich), to reach final concentration of 2.5% and 5%.

NIH3T3 Cell Culture and Treatment
NIH3T3 cells were cultured in complete medium containing DMEM, 10% FBS and 1% Penicillin-Streptomycin in 5% CO2, 37°C, humidified incubator. Upon reaching 80% confluence, NIH3T3 cells were dissociated with 0.05% Trypsin-EDTA and sub-cultured. After gaining enough cell numbers, the NIH3T3 cells were seeded in 96-well or 24-well plate for further assays.

Cytotoxicity Test
Cytotoxicity test was performed using Na,30-[(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzenesulfonic acid hydrate (XTT) Cell Proliferation Assay Kit (Cayman, Ann Arbor, MI, USA), to measure viable NIH3T3 cells as described previously.(10) Five thousand NIH3T3 cells were seeded in 96-well plate overnight in complete medium. The cells were then rinsed with Phosphate-buffered Saline (PBS) and treated with starvation medium; starvation medium and 0.037% H2O2; starvation medium and 2.5% CCBE; or starvation medium and 5% CCBE for 24 or 48 h. Treated cells were added with MTT reagent and incubated in a 37°C incubator for 4 hours. Proliferation was determined from the reduction of tetrazolium into insoluble formazan product by mitochondria of viable NIH3T3 cells. Absorbance was measured with a microplate reader at 595 nm.

Scratch Assay
Scratch assay was performed to measure the cell migration. Fifty thousand NIH3T3 cells were seeded in 24-well plate for 24 h in complete medium. Then the scratch was made on each well using micropipette yellow tips to create 1 µm gap of the cell culture. The cells were then rinsed with PBS and treated with starvation medium; starvation medium and 10% FBS; starvation medium and 2.5% CCBE; or starvation medium and 5% CCBE for 24 or 48 h. The gap width of each well was analyzed and documented under an Axio Vert inverted light microscope (Carl Zeiss, Oberkochen, Germany).

Statistical Analysis
Analysis of Variance (ANOVA) with Least Significant Difference Post-hoc was conducted to point out the difference of each group. The statistical analysis was performed using SPSS Statistics Version 20.0 (IBM Corp., Armonk, NY, USA). The significancy was set at p-value<0.05.

Results

CCBE was Not Cytotoxic for NIH3T3 Cells
In starvation medium, NIH3T3 cells grew >3-folds from 5,000 to 17,254 cells in 24 h and >4-folds from 5,000 to 20,834 cells in 48 h (Figure 1). Meanwhile addition of 0.037% H2O2, number of NIH3T3 cells were significantly (ANOVA, p=0.000) decreased in 24 h, only 880 viable NIH3T3 cells remained. The cell numbers were continuously decreased in 48 h. When the NIH3T3 cells were treated with 2.5% or 5% CCBE for 24 and 48 hours, the numbers of viable NIH3T3 cells were almost similar with the numbers of NIH3T3 cells in starvation.
**CCBE Induced Migration of NIH3T3 Cells**

As shown in Figure 2, starvation medium had low induction of NIH3T3 cells migration. The widths of scratched areas remained large after 24 h (728.24±36.41 µm) and 48 h (681.08±34.05 µm) (Figure 3). When 10% FBS was added the migration was increased as shown by significant (ANOVA, p=0.000) Table 1 decrease of the widths of scratched areas after 24 h (170.18±8.51 µm) and 48 h (27.03±1.35 µm). Meanwhile 2.5% and 5% CCBE showed significant decrease of the widths of scratched areas. As shown in Figure 3, after 24 h and 48 h, the average widths of 2.5% and 5% CCBE-treated scratched areas were 235.68±22.79, 50.36±5.29, 229.95±23.01, 27.68±2.83, respectively.

**Discussion**

Current results showed that treatment of 2.5% CCBE and 5% CCBE for 24 h and 48 h were not cytotoxic for NIH3T3 Cells. Numbers of 2.5% and 5% CCBE-treated viable NIH3T3 cells were almost similar with the numbers of...
NIH3T3 cells in starvation medium merely at 24 h and 48 h. Hence, 2.5% CCBE and 5% CCBE could be potential for further assay. Similar in vitro study with fibroblast/NIH3T3 cell has not been reported, but a research in green coffee beans extract showed that 15% extract was not not cytotoxic, moreover it could induce proliferation of fibroblast.(11)

The basic migration cycle includes expansion, formation of stable attachments, translocation of cells, release of adhesions and retraction in other parts of the cell.(12) The migration is an important factor for tissue regeneration of various part of human.(10) In wound repairment, the purpose of this displacement is to bring the two sides of the open wound area together. The result of joining the two sides of the wound area (13), causing the formation of new connective tissue.(14) Migration of fibroblasts affect the wound healing process in wound area.(15,16) The migration of NIH3T3 cell/fibroblast was induced significantly by 2.5 and 5% CCBE, shown by reduction of scratched area widths. The migration was observed at 24 h treatment of 2.5 and 5% CCBE and getting higher after 48 h treatment.

There were some reports related to wound healing-related cell migration induced by herbal extracts, such as Ocimum gratissimum extract (17) and Coccinia grandis extract.

Although CCBE has shown its potential in inducing cell migration, the molecular mechanism induced by CCBE should be further explored. With the advancement of molecular medicine, scientific evidence of medicine from natural resources could be pursued, so that all potential and mechanism could be disclosed. The are some reports based on genome-wide expression monitoring of genes by microarray analysis, for example Si-Jun-Zi decoction.(18) The results gave insight on its prescription and intestinal wound restitution. In regards to CCBE, further exploration of molecular mechanism should be pursued.

**Conclusion**

Since both 2.5% and 5% CCBE are potential in inducing migration of fibroblast (NIH3T3 cell) and do not
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induce cytotoxicity, the CCBE could be potential as an agent for wound healing. Further investigation should be explored.

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Authors Contribution

YA and FS were involved in concepting and planning the research, FS and LA performed the data acquisition/collection, BC and LA calculated the experimental data and performed the analysis, BC and LA drafted the manuscript and designed the figures, YA and FS aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

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