



EVALUATION OF CYTOTOXIC ACTIVITY OF COMBINATION HONEY AND *ALOE VERA* IN NIH3T3 FIBROBLAST CELL LINES AND ITS EFFECT ON CELL VIABILITY

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ABSTRACT

Alternative treatments can be used singly or in mixture to treat and prevent disease. Honey and *Aloe vera* are natural ingredients that have anti-microbial, anti-inflammatory, antioxidant properties that are used to treat wounds. Animal studies have shown that giving honey and *Aloe vera* can speed up wound healing time. This study aims to examine the cytotoxic effect of the combination of honey and *Aloe vera* on the NIH3T3 cell line and its impact on cell viability. This research is a pure experiment using the post-test only design with control group design. The cytotoxicity test was on NIH3T3 fibroblast cells using the MTT Assay method to determine the inhibitory concentration (IC₅₀) value. Cell viability was calculated using the MTT Assay method. The combination is divided into 5 concentrations, combination 1 (2% honey and 500 µg/mL (0.005%) *Aloe vera*), combination 2 (1.5% honey and 250µg/mL (0.0025%) *Aloe vera*), combination 3 (1% honey and 125 µg/mL (0.00125%) *Aloe vera*), combination 4 (Honey 0.5% and 50µg/mL (0.0005%) *Aloe vera*), combination 5 (Honey 0.1 % and 0.5µg/mL (0.00005%) *Aloe vera*). The combination of honey and *Aloe vera* showed inhibition below 13.87% with an IC₅₀ value of 6.58%. Combination honey and *Aloe vera* on average was able to increase the viability of fibroblast cells above 86.13%. Combination 4 and 5 can increase cell viability 96.50% and 99.80%. The combination of honey and *Aloe vera* does not cause cytotoxic effects on NIH3T3 fibroblasts cell line and can increase the viability of fibroblasts.

Keyword: cytotoxic; combination honey and *aloe vera*; cell viability; in vitro

INTRODUCTION

Wounds are disturbances in the continuity of the epithelial layer of the skin or mucosa. Wounds according to their etiology are classified into acute and chronic wounds (Whitney, 2005). Acute wounds heal in a short time with proper treatment. Chronic wounds take longer because the wound healing process does not develop normally, mainly because of the accompanying pathological conditions such as diabetes (Demidova-Rice et al., 2012). A chronic injury is a significant economic burden and results in a profound reduction in productivity for society and the medical system (Järbrink et al., 2017). It is estimated that diabetes ulcer treatment in the United States costs between the US \$ 9 and the US \$ 13 billion and a total cost of US \$ 8659 per patient (Rice et al., 2014).

The goal of wound care should be to consider the effectiveness of the drug used against the patient's financial burden. With the emergence of alternative wound care products, it is hoped that they will become the right choice for treating chronic wounds. According to WHO, alternative treatments can use plant, animal, and mineral substances that can be used singly or in combination to treat and prevent disease (Fink, 2002). Several ingredients available in nature such as honey and *Aloe vera* are reported to be used in the management of wound healing (Dorai, 2012).

Honey has been known as medicine since centuries ago to treat fever, pain, and treat wounds. Honey has a low pH, hydrogen peroxide (H₂O₂) activity, and various phytochemicals that are

useful for antibacterial and antioxidant activity. The content of vitamin C and flavonoids plays a role in promoting new tissue and increasing the proliferation of fibroblast cells in vitro (Kabbash et al., 2018; Stagos et al., 2018). Meanwhile, Günes and Eser stated that the healing time of honey was four times faster than the methoxy - diaminoacridine group in pressure ulcer patients (Yapucu Güneş and Eşer, 2007). The sugar content acts as an osmolarity which can inhibit the growth of various microorganisms (Kwakman and Zaat, 2012).

Aloe vera is a herbal medicinal plant with biological activities such as anti-microbial, anti-cancer, anti-inflammatory, and immunomodulatory properties that can be used to treat acute and chronic wounds (Kwon et al., 2011). *Aloe vera* contains various active compounds such as polysaccharides, vitamins, amino acids, phenols which play a role in the angiogenesis process (Rahman et al., 2017). Angiogenesis is an important factor in wound healing remodelling. The anti-microbial and anti-inflammatory substances contained in it can prevent excessive TNF- α and IL-6, thereby accelerating the inflammatory phase of wound healing (Bainbridge, 2013). Based on the above, it encourages researchers to evaluate the cytotoxic effect of the combination of honey and *Aloe vera* and the effect of its viability on NIH3T3 fibroblasts. The urgency of the study to assess the combination of honey and *Aloe vera* so that it becomes an option or alternative to natural ingredients that can be used as wound care

METHOD

Research materials

Forest honey, *Aloe vera*, NIH3T3 fibroblast cells (obtained from the Laboratory of Pharmacology and Therapy, Universitas Gadjah Mada), doxorubicin, RPMI 1640 media powder, 10% Fetal Bovine Serum (FBS), Amphotericin B, Streptomycin Penicillin (Penstrep), Trypsin EDTA 0.25%, Sodium Dodecyl Sulfate (SDS) (Gibco®), Sodium bicarbonate (Nacalai Tesque), Phosphate Buffer Saline (PBS) (Invitrogen®), yellow MTT reagent ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Bio Basic Inc.®).

Preparation of a combination of honey and *Aloe vera*

The honey used is obtained from the Kitamura Clinic Pontianak from the Putusibau forest of West Kalimantan. Honey is dissolved in Dulbecco's Minimal Essential Medium (DMEM, Gibco). *Aloe vera* was obtained from the Kulon Progo Agricultural and Rural Training Center, Yogyakarta. *Aloe vera* extract was made at the Laboratory of Pharmacology and Therapy, Universitas Gadjah Mada. Furthermore, the two ingredients are made into a combination divided into 5 concentrations, namely combination 1 (2% honey and 500 μg / mL (0.005%) *Aloe vera*), combination 2 (1.5% honey and 250 μg / mL (0.0025%) *Aloe vera*), combination 3 (1% honey and 125 μg / mL (0.00125%) *Aloe vera*), combination 4 (Honey 0.5% and 50 μg / mL (0.0005%) *Aloe vera*), combination 5 (Honey 0.1% and 0.5 μg / mL (0.00005%) *Aloe vera*).

Cytotoxic test using the MTT Assay method

NIH3T3 fibroblasts were inserted into the 96 well microplate each 100 μL / well as much as 1×10^4 . Cells were incubated for 24 hours under starvation. Treated with each concentration of the combination then incubated again for 24 hours. The well was added with 100 μL of new media and 10 μL of MTT reagent (10 μL / 100 μL per well), then incubated for 4-6 hours in a CO₂ incubator at 37°C. Each well was added with 100 μL of 10% sodium dodecyl sulphate (SDS) in 0.01% HCl. Incubated at room temperature for 12 hours or overnight. The microculture wells were then read for their absorbance using an ELISA reader at a wavelength of 595 nm

Cell Viability Test

The percentage of the number of living cells can be calculated by the MTT Assay method. The results were read using a microplate reader and calculated using the formula for the percentage of cell viability = (Treatment absorbance - Media absorbance) / (Control cell absorbance - Media absorbance) x 100%.

RESULTS AND DISCUSSION

Evaluation of the cytotoxic combination of honey and *Aloe vera* on NIH3T3 fibroblast cells.

After an intervensi, it can be seen that percentage of combination honey and *Aloe vera* is below 13.87% and the IC50 value is 6.581% x 104. Meanwhile, using *Algisite-M*[®] as a positive control the inhibition value was 33.08%.

Table 1.
 Value of percentage of inhibition of the combination of honey and *Aloe vera*

Testing material	Percentage of cell proliferation inhibition			Inhibition average	IC50 value
	Repeat 1	Repeat 2	Repeat 3		
	Inhibition (%)	Inhibition (%)	Inhibition (%)		
Combination 1	9.36	12.64	19.61	13.87	6,58%
Combination 2	12.64	10.94	16.59	13.39	
Combination 3	6.24	11.56	7.99	8.59	
Combination 4	-2.79	7.39	5.88	3.49	
Combination 5	-2.97	0.54	3.02	0.2	
<i>Algisite-M</i> [®]	26.93	31.43	40.87	33.08	

Observation of NIH3T3 fibroblast cell viability

Table 2.
 The percentage value of NIH3T3 fibroblast cell viability after treatment

Testing material	Mean cell viability (%) ± SD	p value
Combination 1	86.13 ± 5.23 ^a	0.000*
Combination 2	86.60 ± 2.89 ^a	
Combination 3	91.40 ± 2.70 ^a	
Combination 4	96.50 ± 5.49 ^{ab}	
Combination 5	99.80 ± 3.00 ^{ab}	

*= ANOVA test; a = p <0.05, ANOVA test continued with Post hoc LSD, compared with the positive control group; b = p <0.05, ANOVA test continued with Post hoc LSD, compared with combination groups 1, 2 and 3.

The data shown in Table 2 shows that giving a combination of honey to each group can increase cell viability. There was an increase in the average percentage of cell viability in each group compared to the control group using *Algisite-M*[®]. In the normal wound healing process, fibroblasts will migrate and proliferate in a balanced manner to replace damaged cells or tissues to maintain the survival of an organism (Bainbridge, 2013). Fibroblasts play an important role in the proliferation phase by increasing various growth factors such as VEGF-A, TGF-β, and CTGF. Besides, fibroblasts will also migrate and proliferate to the wound area and then differentiate into myofibroblasts to increase wound contractile power, maturation of granulation tissue supports signal angiogenesis and epithelialization. The fibroblasts used in this study were NIH3T3 fibroblasts. In this study, NIH3T3 fibroblasts were selected because fibroblasts are present in the matrix and connective tissue of the body and are widely used to

determine cellular cytotoxicity and genotoxicity of various formulations (Coradeghini et al., 2013). The combination concentration of honey and *Aloe vera* used in our selected cytotoxicity studies depends on the studies conducted in the literature (Negahdari et al., 2017; Rizqi et al., 2019).

In vitro studies, the MTT Assay is used to determine cytotoxicity in cells. This test is an important test method in assessing the biocompatibility of a new formula and ensuring the material used is safe for research both in animal studies so that it does not pose a risk or cause harm (LI et al., 2015). The results of this study indicate that the combination of honey and *Aloe vera* for 24 hours has an inhibitory value of less than 13.87% against NIH3T3 fibroblast cells with an IC₅₀ value of 6.581% x 10⁴ (Table 1). There were no data on the IC₅₀ value of the combination of honey and *Aloe vera* on fibroblasts in the literature that we found. The results of several previous studies, administration of honey, showed that an inhibitory concentration of 50% was obtained IC₅₀ ranging from 3.1mg/mL to 44.4mg/mL and depending on the type of honey tested (Leong et al., 2012). Honey testers with an inhibitory value of more than 50% at concentrations above 3% (Rizqi et al., 2019). Higher concentrations of honey can cause contamination problems and inhibit cell proliferation (LI et al., 2015; Munshi, 2014). Meanwhile, for *Aloe vera*, the IC₅₀ inhibition value was 285.50 µg/mL (Candoken et al., 2018). From some of the literature above, it shows that the combination of honey and *Aloe vera* in this study does not cause a strong toxic effect seen from the inhibition value which is less than 13.87%.

Cell viability is the number of cells that are still alive from the total cells in a sample. In this study, giving a combination of honey and *Aloe vera* increased the viability of fibroblast cells. In Table 2, a significant value is obtained between the treatment group compared to the control group. Administration of a combined concentration of 4 (0.5% honey and 50 µg/mL *Aloe vera*) and five concentration (0.1% honey and 0.5 µg/mL *Aloe vera*) significantly increased cell viability compared between groups. *Aloe vera* has the potential to increase cell viability [15]. *Aloe vera* is nontoxic and can increase the proliferation of fibroblasts and improve cell viability with a range of values ranging from 90% to 112% (Curto et al., 2014). Increasing the value of cell viability also occurs along with the increase in the concentration of *Aloe vera* extract, so that a greater concentration of *Aloe vera* extract is needed to maintain or increase cell viability (Gontijo et al., 2013). However, in this study, the combination of *Aloe vera* with the lowest concentration showed the highest viability (Table 2).

In this study also using *Algisite-M*[®] as a control group, is a standard dressing that is often used to maintain moisture, absorb exudates and stimulate cell proliferation. *Algisite-M*[®] dressings are nontoxic and can maintain the viability of 89.5% in keratinocyte cells (Paddle-Ledinek et al., 2006). The calcium content contained in *Algisite-M*[®] dressings can trigger basic cellular activities such as adhesion, cell movement, proliferation and also cell differentiation (Paddle-Ledinek et al., 2006). Honey is used as a topical wound healing agent because it has antimicrobial, antioxidant and anti-inflammatory properties. Besides, honey with the right concentration can create a moist environment, which is useful for stimulating an increase in growth factors, stimulating the proliferation and migration of fibroblasts. *Aloe vera* has glycoprotein and polysaccharide components which significantly increase the proliferation and migration of fibroblast cells and affect EGF expression and collagen synthesis by increasing cell metabolism (Kabbash et al., 2018). The combination of these two materials is a new alternative dressing for wound care because it has a low cytotoxic effect and can increase the viability of fibroblast cells compared to the control group.

CONCLUSION

The combination of honey and *Aloe vera* has a low cytotoxic effect on NIH3T3 fibroblasts with an IC50 value of 6.58%, and is able to increase cell viability to 99.80%.

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