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Cytotoxic Effect of Essential Oil from Cinnamon (*Cinnamomum burmannii*) Bark on Rat Bone Marrow Mesenchymal Stem Cells: In Vitro Study

Budiastuti[1], Niken Dwi Lestari[2], Mustofa Helmi Effendi[3]* , Arimbi[4], and Hani Plumeriastuti[4]

[1]Doctoral program on Faculty of Pharmacy, Universitas Airlangga.
[2]Undergraduate student on Faculty of Veterinary Medicine, Universitas Airlangga.
[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga.
[4]Departement of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga
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***Corresponding author**: Mustofa Helmi Effendi; Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Email : mheffendi@yahoo.com

ABSTRACT
The aim of this study was to determine the cytotoxicity properties of essential oil from Cinnamon (*Cinnamomum burmannii)* bark on rat bone marrow mesenchymal stem cells (rBMSCs). This research was an experimental laboratory with The Post Test Only Group as a research design. Essential oil from Cinnamon (*Cinnamomum burmannii)* bark obtained from steam distillation method and essential oil was made in a series dilution with a concentration of 0,5%, 0,25%, 0,125%, 0,0625% and 0,0312%. Furthermore, the cytotoxicity test on rBMSCs using MTT assay. Microplate containing rBMSCs that had been exposed to five concentrations was incubated in a 5% CO_2 incubator with 37 °C for 24 hours. The result of MTT assay can be seen from the absorbent solution formazan crystal through ELISA reader with the specific wavelength of 595 nm which produces data in the form of Optical Density (OD). The result research showed that the rBMSCs cell life percentage in 0,5%, 0,25%, 0,125%, 0,0625% and 0,0312% of concentration respectively were 12,8%, 18,3%, 21,7%, 26,5% and 32,5%. Probit analysis is used to determine the LC_{50} of essential oils from Cinnamon (*Cinnamomum burmannii)* bark. It can be concluded that the smaller the concentration of essential oils given to rBMSCs, the higher the percentage of cell life. In this study, the concentration of essential oils which can kill 50% of rBMSCs was 0,004%.

Keywords: Cytotoxicity, *Cinnamomum burmannii*, essential oil, rBMSCs, MTT assay

Correspondence:
Mustofa Helmi Effendi
Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga.
Email : mheffendi@yahoo.com

INTRODUCTION
The bark of various types of cinnamon is one of the most important and popular spices used throughout the world, not only for cuisine but also for traditional and modern medicine. In total about 250 species have been identified among the genus of cinnamon and these plants are spread throughout the world [1]. Cinnamon species that native plant from Indonesian is *Cinnamomum burmannii* or commonly known commercially as Cinnamon Koerintji [2, 3, 4]. Various studies have been conducted to reveal more in the medical value of cinnamon than as a spice. The uses of traditional cinnamon are for astringents, disinfectants and antispasmodics [5]. The research conducted in India found that in vitro incubation of cinnamon extract caused an increase in insulin release [6]. Essential oil from cinnamon bark is generally used at a dose of 0.05 to 0.2 g daily as an antidiabetes drug [7].
According to Ravindran, *et al.* (2004) stated that the largest content in cinnamon essential oil is cinamaldehyde, around 51-76% [8]. Adawiyah, *et al.,* (2015) reported that the amount of cinnamaldehyde for consumption not more than 700μg / kg [9], showed rats given cinnamon extract in doses of 0.1-2.0g / kg orally resulted in damage to the liver and kidneys. Another researcher reported on their research that on bioassay screening of essential oils from 4 kinds of medicinal plant extracts showed that cinnamon oil (*Cinnamomum zeylanicum*) has very strong cytotoxic properties, with the value from lethal concentration (LC_{50}) of 0.03 mg /ml [10].
Essential oil from cinnamon bark which has been increasingly known for it is medical potential based on previous research, cytotoxic testing is needed to determine its safety. A toxicity test is a test used to determine safe levels of drugs or other chemicals carried out with experimental animals or other biological materials [11]. However, the cytotoxicity effect of essential oils on rat bone marrow mesenchymal stem cells has never been done. According to Griffin, *et al.,* (2010) showed that bone marrow-derived mesenchymal stem cells (BMSCs) are multipotent stem cells and are important cell sources for engineering purposes in terms of cell therapy and tissue repair, these cells are believed to be more sensitive than other cells [12]. Therefore, this study was conducted to determine the cytotoxicity effects of essential oils from Cinnamon (*Cinnamomum burmannii)* bark on rat bone marrow mesenchymal stem cells and to determine the value of lethal concentration 50 (LC_{50}).

MATERIAL AND METHODS
The study was conducted at the Stem Cell Research and Development Center, Campus C Airlangga University, Mulyorejo, Surabaya, East Java 60115. The distillation process of essential oil from Cinnamon (*Cinnamomum burmannii)* bark was carried out at PT. Heptasari Unggul, Jl. Demak No. 289, Surabaya, East Java 60179.
This study used rat bone marrow mesenchymal stem cells (BMSCs) which is obtained from the Stem Cell Research and Development Center, Airlangga University. The primary cell culture rat BMSCs was stored in a deep freezer at -80 °C in a freezing medium. First, the cell must go through the thawing process. The cells were pipette into a conical tube that already contained αMEM media,

then centrifuged for 5 minutes at 2400rpm. The media is removed and replaced with the new αMEM media, then centrifuged again at the same speed. This washing process is done twice to remove the remaining DMSO [13]. The formed supernatant is thrown into the glass jar so that only cells in the form of deposits remains on the bottom of the tube or called pellet. After that pellet are diluted with 10ml αMEM, then taken using a filler pipette and placed in a Ø10 cm petri dish to cultivated the cell.

The rat bone marrow mesenchymal stem cells (rBMSCs) were used in 96 well microplates. This study consisted of 5 treatment groups with 6 repetitions, K (-) or media controls which only contained cell growth media (α-MEM) and K (+) or cell controls containing rBMSCs and α-MEM media. The treatment group consisted of, group giving essential oil 0.5%, group giving essential oil 0.25%, group giving essential oil 0.125%, group giving essential oils 0.0625%, and group giving essential oils 0.0312%. The treated cells were then incubated for 24 hours in a CO_2 incubator at 37 ° C. *3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide* (MTT) dissolved in Phosphate Buffered Saline (PBS) 5 mg / ml then added to the microplate 96-well as much as 10µl per each well, then incubated again for about 4 hours at 37 °C. All media in the well and test material are discarded. Then, each well is added to 50µl -100µl of DMSO (Dimethylsulfoxide).

Formazan crystals formed due to the reduction of MTT salts by dehiderogenase enzymes in living cells are then dissolved with DMSO. The absorbance value is read by ELISA reader with a wavelength of 595nm. The research data obtained in the form of Optical Density (OD) values were analyzed using the ANOVA test and calculated the LC50 value.

RESULTS AND DISCUSSION

The results which viewed throught ELISA reader with a wavelength of 595nm in this study were in the form of Optical Density (OD), as shown on table 1. The OD value is obtained through the color changes which produced by mitochondrial activity, where the dehyderogenase enzyme in living cells is able to reduce the yellow MTT salt to form blue / purple formazan crystals. The darker colors were produced, the higher the OD value. Optical density values represent the number of living rBMSCs so that the greater the optical value of density shows the greater the number of living cells.

In figure 1, showed the percentage of living cells of rat BMSCs after being treated with essential oil from Cinnamon (*Cinnamomum burmannii)* bark with different concentrations. The percentage of living rat bone marrow mesenchymal stem cell after being given essential oil from cinnamon bark with a concentration of 0,5% was as much as 12,886% . At a concentration of 0,25% , the percentage of living cells was as much as 18,364%. At a concentration of 0,125% , the percentage of living cells was as much as 21,768%. At a concentration of 0,0625% , the percentage of living cells was as much as 26.554%. At a concentration of 0,0312% , the percentage of living cells was as much as 32.551%.

Observations using a microscope showed that the concentration of essential oil from Cinnamon (*Cinnamomum burmannii)* bark affected the number of living cells. The smaller the concentration of essential oil from cinnamon bark given, the higher the number of living cell. In figure 2. showed that the concentration of 0,0312% produces the highest percentage of living cell when compared with other concentrations.

Probit analysis was done to determine the concentration of lethal cell 50 (LC_{50}) on rat bone marrow mesenchymal stem cell. The concentration of essential oil from Cinnamon (*Cinnamomum burmannii)* bark which can kill 50% (LC_{50}) of rat bone marrow mesenchymal stem cell which tested is 0,004%.

The highest percentage of rat bone marrow mesenchymal stem cell (rBMSCs) life is 32,5% which occurs at a concentration of 0,312% while the lowest percentage of cell life occurs at a concentration of 0,05% which is 12,8%. This shows that the lower the concentration of essential oil from cinnamon bark that is given, the higher the percentage of the life of rBMSCs. The higher the concentration consist the higher the active substance. Cinnamaldehyde and eugenol are the most active ingredients contained in cinnamon. The presence of cinnamaldehyde in the essential oil of *C. burmannii* has been responsible for the observed cytotoxicity. The cytotoxicity of cinnamaldehyde acid has been reported previously [14]. Cinnamaldehyde is toxic to larvae by entering the body of the larvae through the respiratory system which results in nerve disorders and damage to the respiratory system, causing death [15].

The mesenchymal stem cells of rat bone marrow after being given with cinnamon essential oil with a concentration of 0.5% and then incubated in 5% CO2 incubator at 37 ° C for 24 hours produced the smallest percentage of cell life. This situation seems to be in accordance with the opinion of Adriani, *et al.,* (2010) stated that cinnamaldehyde has a toxic mechanism that can inhibit energy metabolism in cells. This causes the cell to fail to adapt to the material [16].

In this study the graphic percentage of living cell was decreased, that in cinnamon essential oil with a concentration of 0,0312%, 0,0625%, 0,125%, 0,25% and 0,5% showed the life of stem cells of rat bone marrow in succession 32,5%, 26,5%, 21,7%, 18,3% and 12,8% respectively. This result shows compatibility with the previous theory which state that the toxicity of a material is directly propotional to exposure. Exposure of material has a determining factor, that was the consentration of the material [17]. This shows that all concentrations of essential oils of *C. burmannii* have the same effect, namely decreasing the percentage of cell life and the greater the concentration given the smaller the percentage of cell life. This result seems similar to research, on the toxicity of *C. burmannii* essential oil on fibroblast cell culture, that the graph of the percentage of the life of fibroblast cells increases when the amount of essential oil concentration given decreases [18]. An experimental cytotoxicity test of cinnamon oil on WiDr cells, and the highest percentage of cell viability was achieved using the smallest concentration [19].

Bioassay screening of essential oils in *Cinnamomum zeylanicum* conducted by Sharififar, *et al.,* (2009) showed a strong cytotoxicity potential of essential oils , which is have value of LC_{50} on 0.03mg / ml [10]. In essential oils from *Cinnamomum burmannii* in this study, after being analyzed by probit analysis showed LC_{50} values of 0,004%. This result is much smaller than previous research. The use of stem cell mouse marrow is possible to be one factor because these cells are more sensitive than other cells.

An essential oil that is derived from Cinnamomum cassia stem bark, coumarin has been successfully isolated. Due to the potential for high levels of coumarin, it may cause hepatotoxicity [20]. Trans-cinnamaldehyde (TCA) has been identified as one of the bioactive compounds in *Cinnamomum burmannii*, in research showing that trans-cinnamaldehyde can inhibit cell proliferation and induce apoptosis. The mechanism of apoptosis by cinnamaldehyde is the production of Reactive Oxygen Species (ROS). Cinnamaldehyde from *Cinnamomum casia* is a potent inducer of apoptosis and that it transduces the apoptotic signal via ROS generation, thereby inducing mitochondrial permeability transition (MPT) and cytochrome release to the cytosol [21]. Cinnamaldehyde induce cell death was characterized by changes in nuclear morphology, DNA fragmentation, and cell morphology [22]. Treatment with cinnamaldehyde was caused by a rapid loss of mitochondrial transmembrane potential, stimulation of ROS production [23], the release of mitochondrial cytochrome c into cytosol and subsequent induction of procaspase-9 and procaspase-3 processing. Thus in this study, cinnamaldehyde from *Cinnamomum burmannii* is also likely to induce apoptosis.

CONCLUSION

Essential oil from cinnamon (*Cinnamomum burmannii*) bark at a concentration of 0,5% produces the lowest percentage of living rat bone marrow mesenchymal stem cell and at a concentration of 0,0312% produces the highest percentage of living rat bone marrow mesenchymal stem cell. The concentration of essential oil from cinnamon (*Cinnamomum burmannii*) bark which can kill 50% (LC_{50}) of rat bone marrow mesenchymal stem cell is 0,004%.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Optical Density (OD) result from rat bone marrow mesenchymal stem cell after exposed with essential oil from Cinnamon (*Cinnamomum burmannii)* bark for 24 hours

Treatment	Optical Density (OD) Value					
	1	2	3	4	5	6
Media control	0,095	0,096	0,094	0,098	0,093	0,096
Cell control	0,561	0,565	0,572	0,565	0,597	0,522
Essential oil 0,5%	0,156	0,165	0,151	0,153	0,159	0,156
Essential oil 0,25%	0,181	0,180	0,174	0,193	0,192	0,184
Essential oil 0,125%	0,219	0,196	0,223	0,154	0,198	0,201
Essential oil 0,0625%	0,238	0,215	0,223	0,206	0,211	0,197
Essential oil 0,0312%	0,244	0,253	0,241	0,256	0,242	0,257

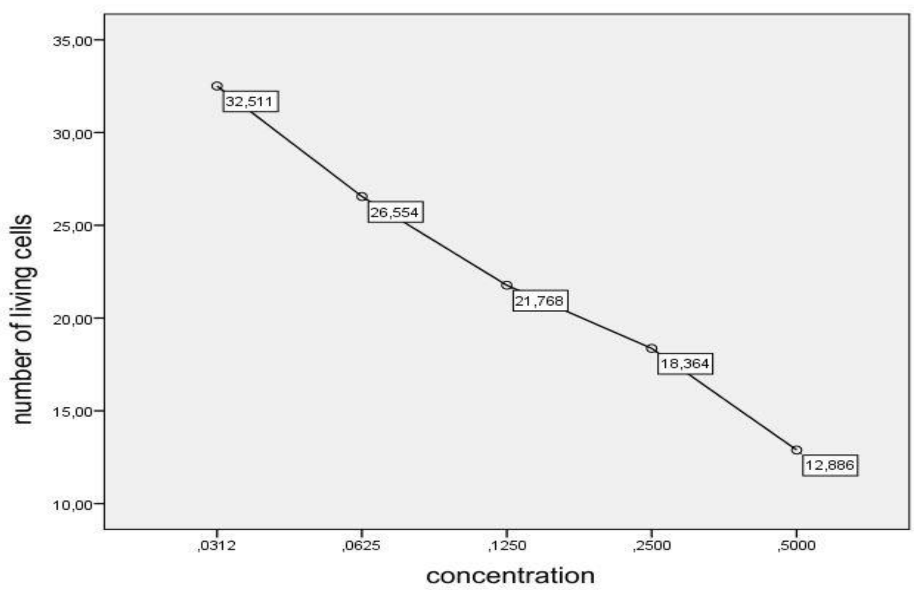

Figure 1. Comparison of the percentage of living rat BMSCs after treated with essential oil from Cinnamon (*Cinnamomum burmannii)* bark with different concentrations.

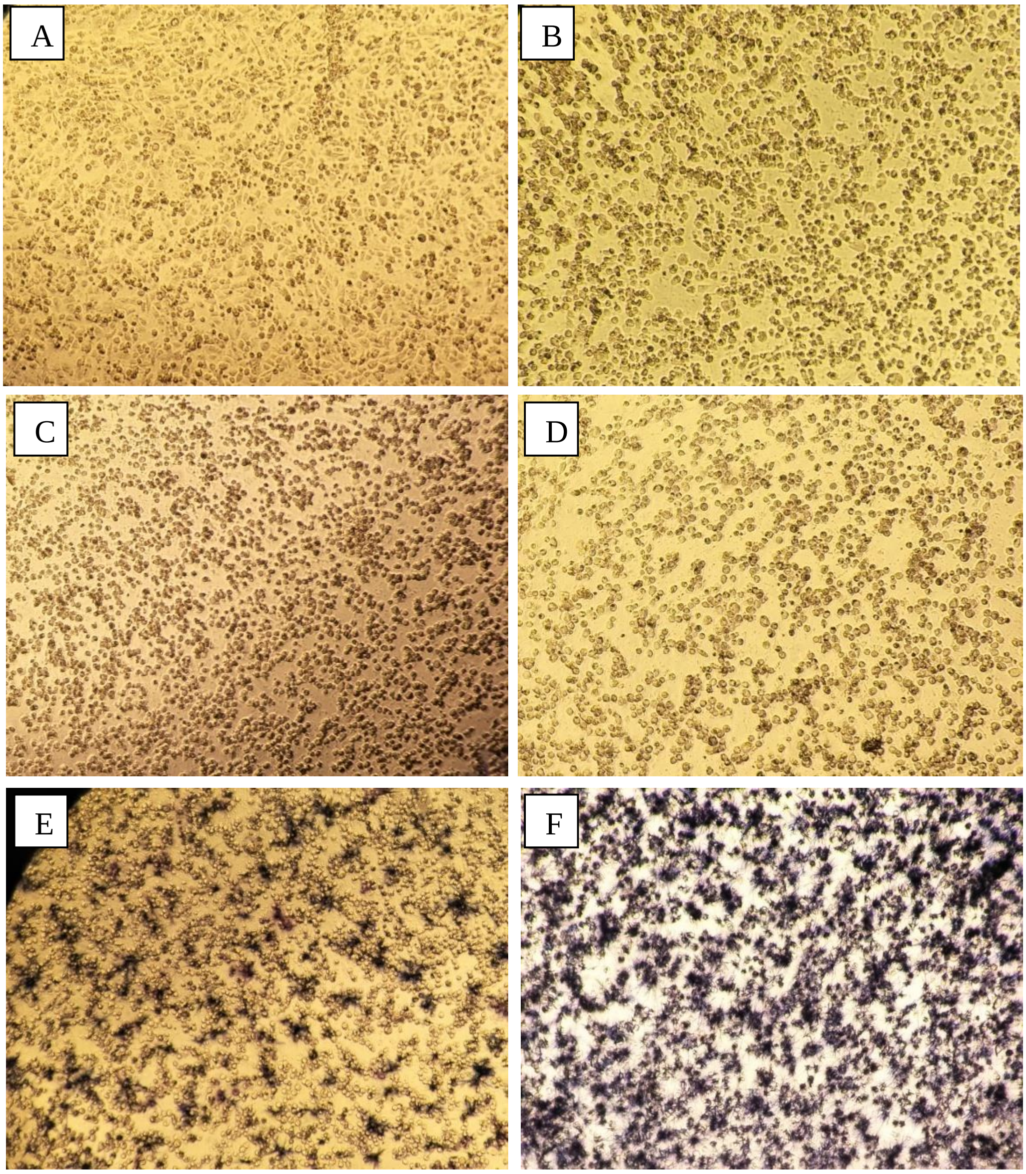

Figure 2. Overview of rat BMSCs after exposed with essential oil from Cinnamon (*Cinnamomum burmannii)* bark, using inverted 100x microscope. A. 0,5% of concentration. B. 0,25% of concentration. C. 0,125% of concentration. D. 0,0625% of concentration. E. 0,0312% of concentration. F. Control cell.