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Cytotoxicity of Eurycomanone and Fargesin in RAW 264.7 Murine Macrophages

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bioactive natural compounds derived from plants are the source for the development of new drugs. Numerous *in vitro* studies have explored the anti-inflammatory effect of eurycomanone and fargesin, derived from *Eurycoma longifolia* and *Flosmagnoliae*, respectively. However, before anti-inflammatory investigation is conducted, it is important to obtain the safe doses of these compounds to ensure the validity of the anti-inflammatory results. Therefore, the present study was

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aimed to investigate the cytotoxicity of eurycomanone and fargesin towards macrophage RAW 264.7. cells to determine the safe doses of these compounds. Different concentrations of eurycomanone and fargesin were subjected to RAW 264.7 cells. The cytotoxicity of the compounds was evaluated by MTT assay and 50% inhibitory concentration (IC_{50}) of these compounds was determined. Morphological changes of RAW 264.7 cells upon exposure to these compounds were also observed. Eurycomanone exhibited its cytotoxic effect by reducing RAW 264.7 cell viability dose-dependently with the IC_{50} of 94.17 µM. Meanwhile, fargesin had slight cytotoxicity towards RAW 264.7 cells with the IC_{50} of 173.5µM. Eurycomanone was more cytotoxic towards RAW 264.7 cells compared to fargesin. In conclusion, eurycomanone and fargesin at concentration up to 25 µM, was not toxic to the RAW 264.7 murine macrophages cells and the findings can be applied in the future anti-inflammatory study.

Keywords: Cytotoxicity; eurycomanone; fargesin; RAW 264.7 cells; IC50.

1. INTRODUCTION

Nature isthe major source of bioactive compounds that are used in both traditional and modern medicines. For the past years, many drugs have been developed directly from bioactive natural compounds and plants are the most explored resourcesfor the development of new drugs and pharmacological studies [1-4].

Drugs originated from plants used in treatment as anti-inflammatory, anticancerous, and analgesic, include acetylsalicylic acid (Aspirin), digoxin, paclitaxel, vincristine and morphine.For instance, Aspirin, is naturally occurring polyphenol that can be found in *Salix sp. (Salicaceae)*. It has been used as an anti-inflammatory drug, whereasPaclitaxel, derived from *Taxus brevifolia* is an anticancer drug [5,6]. Hence, exploring the potential of a plantbioactive compound is paramount in the drug discovery and development.

Eurycoma longifolia is native to Malaysia, Indonesia, Vietnam, Cambodia, Laos, Myanmar and Thailand. Reported pharmacological activities including anticancerous, antimalarial, anti-inflammatory, antioxidant, antimicrobial and aphrodisiac [7]. Most of the studies on *E. longifolia* focus on the anticancerous, anti-inflammatory and male fertility enhancement effect of the active compounds called eurycomanone[7-9].

Bioactive lignan from *Flosmagnoliae*, fargesin is a Chinese herb that is used to treat sinusitis, allergic rhinitis, nasal congestion and headache. Previous studies also indicate that fargesin has anti-inflammatory, anti-allergy, antimicrobial and antidiabetic effects [10-13]. Most of the studies on fargesin derived from *Flosmagnoliae* is on their anti-inflammatory effect [14-17].

Macrophages cells have been frequently used in the studies to elucidate the in inflammation process and anti-inflammation properties of interest compounds [18-24]. However, before any on anti-inflammatory investigation can be carried out, the cytotoxicity of compounds of interest towards macrophage cells need to be evaluated in order to determine the sub-lethal dose.Generally, cytotoxicity of a bioactive compound is indicated when the compound can alter cell morphology or metabolism, affect cell proliferation of induced cell death [25]. Therefore, the aim of this study was to assess the cytotoxicity of eurycomanone and fargesinon RAW 264.7 cells to identify safe dose of these compounds. The findings from this study can be applied in the future anti-inflammatory study.

2. MATERIALS AND METHODS

2.1 Materials and Reagents

Eurycomanone (catalog no.: CFN92008) and Fargesin (catalogno. : CFN98174) were purchased from Wuhan ChemFaces Biochemical Co., Ltd. (Wuhan, Hubei, China). Dulbecco's modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Massachusetts, USA) and Fischer Scientific (Paisley, UK), respectively. Phosphate buffer saline tablet was acquired from Sigma-Aldrich (Missouri, USA). Penicillin-Streptomycin Mixed Solution was purchased from NacalaiTesque, Inc., Kyoto, Japan while trypsin was obtained from Thermo Fisher Scientific (Denmark). All other chemicals were of analytical grade and made available from standard commercial suppliers.

2.2 Cell Culture

Murine macrophages, RAW264.7 cells (ATCC® TIB-71™) were obtained from American Type

Culture Collection (ATCC) (Virginia, United State).Cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 IU mL^{-1} penicillin and 100 g mL^{-1} streptomycin,incubated at 37 °C in a humidified atmosphere with 5% CO_2. The cells were cultured every week according to ATCC protocol.

2.3 Cytotoxicity of Different Concentrations of Eurycomanone and Fargesin

RAW 264.7 cells were treated with different concentrations of eurycomanone and fargesin (1.56 µM, 3.13 µM, 6.25 µM, 12.5 µM, 25 µM, 50 µM, 100 µM and 200 µM) in order to evaluate the cytotoxicity of these compounds on the cells, hence determined the sub-lethal dose of these compounds for further study. RAW264.7 cells were seeded into 96-well plate for 24 hours, then the cells were treated with different concentrations of eurycomanone and fargesin for another 24 hours. After that, the cells were subjected for MTT assay and morphological observation.

2.4 Determination of Cell Viability by MTT Assay

Tetrazolium bromide salt (0.5 mg/mL of stock in PBS) was used to access the cytotoxic effect of eurycomanone and fargesin on the viability of RAW 264.7 cells. Basically, the test is used to assess the mitochondrial NADH-dependent dehydrogenase activity which is proportional to cell viability. After treatment, 100 µL of 0.5 mg/mL of tetrazolium bromide salt were added to the cells in the 96-well plate. Next, the plate was incubated for 3 hours at 37 °C in a humidified atmosphere with 5% CO_2. Then, the MTT solution was removed carefully and the insoluble formazan was dissolved in 100µL of dimethylsulfoxide (DMSO) to each well. The absorbance was measured at 570 nm using Multiwell micro- plate reader (Synergy HT, Bio-Tek Instruments, Inc. Vermont, USA).Theuntreated sets were also run under identical conditions and served as control.

2.5 Morphological Observation

Tetrazolium bromide salt (0.5 mg/mL of stock in PBS) was used to assess the percentage cell viability. In short, the cells were seeded in 96-well plate and incubated at 37 °C in a humidified atmosphere with 5% CO_2overnight. After that, the medium contained either of H_2O_2 (0.125, 0.25, 0.5, 1, 2, 4 mM) or eurycomanone (0µM to 50µM) or combination of $H_2O_2$and eurycomanone were added once the previous medium was removed. 100 µL of Tetrazolium bromide salt (0.5 mg/mL of stock in PBS), the MTT solution, was added to the 96-well plate. Next, the plate was incubated for 3 hours at 37 °C in a humidified atmosphere with 5% CO_2. Then, the MTT solution was removed carefully and the insoluble formazan was dissolved in 200µL of dimethyl sulfoxide (DMSO) to each well. The absorbance values were measured at 570 nm using Multiwell micro- plate reader (Synergy HT, Bio-Tek Instruments, Inc. Vermont, USA).Theuntreated sets were also run under identical conditions and served as control.

2.6 Determination of IC50 of Eurycomanone and Fargesin

IC_{50} is the lethal dose of the compounds that is needed to kill half of the RAW 264.7 cells' population. Cell viability's results from the MTT assay were used to determine the IC_{50} of eurycomanone and fargesin. Percentage mean values of cell viability were transferred into Prism 8 (Graphpad) and dose-response curve of the compounds were generated and analysed. Dose-response curves were parameterized by the log(concentration compounds) against response, which the response is the percent cell viability when compared to control. The IC_{50} values were calculated using the non-linear regression analysis (curve fit).

2.7 Statistical Analysis

The results were expressed as mean ± standard deviation (SD) from at least three independent experiments between different concentrations of eurycomanone and fargesin. Student's t-test was used to determine the significant difference between different concentrations of compounds with the control (0.5% DMSO). Microsoft Excel 2016 and Prism 8.0 (GraphPad) software were used for statistical analysis and determination of IC_{50} of the compounds, respectively. $p < 0.05$ indicated significant differences.

3. RESULTS

3.1 Cell Viability of RAW 264.7 Cells upon Subsequent Exposure to Eurycomanone

Eurycomanone, quassinoids from *E. longifolia*, with concentration ranging from 0 µM to 200 µM concentration was subjected to macrophage RAW 264.7 cells and the cell viability was

assessed using MTT assay. Result in Fig. 1B shows that eurycomanone reduces RAW 264.7 cells viability dose-dependently. There was a dramatic decrease in RAW 264.7 cells viability from 100 µM to 200 µM. RAW 264.7 cells treated with 50 µM and 200 µM eurycomanone were statistically significant to control (0.5% DMSO) with the cell viability of 68% and 25%, respectively.

3.2 RAW 264.7 Cells Morphology upon Exposure to Eurycomanone

Microscopic digital camera was used to observe the morphology of RAW 264.7 cells. The images were recorded and digitized from the TopView application. Fig. 1c portrayed the RAW 264.7 cells upon exposure to distinct concentrations of eurycomanone. The macrophage cells morphology in DMEM and control (DMSO) portrayed a mixture of small, rounded, flattened and expanded cells. There were no signs of cell shrinkage and the cells were attached to the surface. Macrophage cells treated with 1.56 and 3.13 µM eurycomanonewere no obvious difference of morphological characteristic than control cells. Macrophage cells treated with 6.25 and 12.5 µM of eurycomanone were more flattened and expanded compared to control cells which were smaller and rounded. There were few cells shrink and floating in media upon exposure to 25 and 50 µM eurycomanone. Treatment with 100 µM eurycomanone significantly changed the structural alterations and reduction cells population. The cells were less rounded, detach from the surface and float in the media.

3.3 IC_{50} of Eurycomanone

IC_{50} of eurycomanone was determined using Prism 8.0 (GraphPad). IC_{50} is the toxic concentration of eurycomanone needed for 50% inhibition of cell viability. Fig. 1D portrayed the dose-response curve of eurycomanone. The IC_{50} of eurycomanone is 94.17 µM, which means that 94.17 µM of eurycomanone was required to kill half of the macrophage's cell population.

3.4 Cell Viability of RAW 264.7 Cells upon Subsequent Exposure to Fargesin

Fargesin, a bioactive lignan from *Flosmagnoliae*, ranging from 0 µM to 200 µM concentration was treated to macrophage RAW 264.7 cells and MTT assay was used to determine cell viability. Fig. 2B shows that fargesin had slight cytotoxic effects on RAW 264.7 cells up to 25 µM concentration with cell viability fluctuating between 90% to 100%. As the fargesin concentration elevated to 50 µM and 100 µM, the macrophage cell viability reduced to 85%. There was a sharp decrease in macrophage cell viability when fargesin concentration was increased to 200 µM. RAW 264.7 cells subjected to 50 µM and 200 µMfargesin were statistically significant to control (0.5% DMSO) with cell viability of 84.7% and 44.5%.

3.5 RAW 264.7 Cells Morphology upon Exposure to Fargesin

Morphology of RAW 264.7 cells were observed under a microscopic digital camera. The TopView application was used to record and digitize the images. Fig. 2B depicted RAW 264.7 cells after exposure to different concentrations of fargesin. Macrophage cells in this Fig. have a rounded and expandable cells which were attached on the surface.

Macrophage cells morphology treated with fargesin doses between 1.56 to 25 µM did not show much difference than control cells. As concentration of fargesin elevated (50 and 100 µM), the cells changed its shape to round and detached. Treatment with 200 µM fargesin resulted in dramatic morphological changes in the cells and decrease in cell population. Cell cytoplasmic shrinkage, reduction in size, and tendency to float in the medium after the treatment were observed.

3.6 IC_{50} of Eurycomanone

Prism 8.0 (GraphPad) was used to determine the IC_{50} of fargesin. It is the lethal concentration of fargesin required to inhibit 50% cell viability. Results in Fig. 2D represent the dose-response curve of fargesin. The IC_{50} of fargesin is 173.5 µM. In other words, 173.5 µM of fargesin was needed to destroy half of the macrophages's cell population.

4. DISCUSSION

Bioactive compounds specifically originate from plants have received major attention in the development of new drugs and pharmacological studies. *E. longifolia* and *M. fargesii* have been

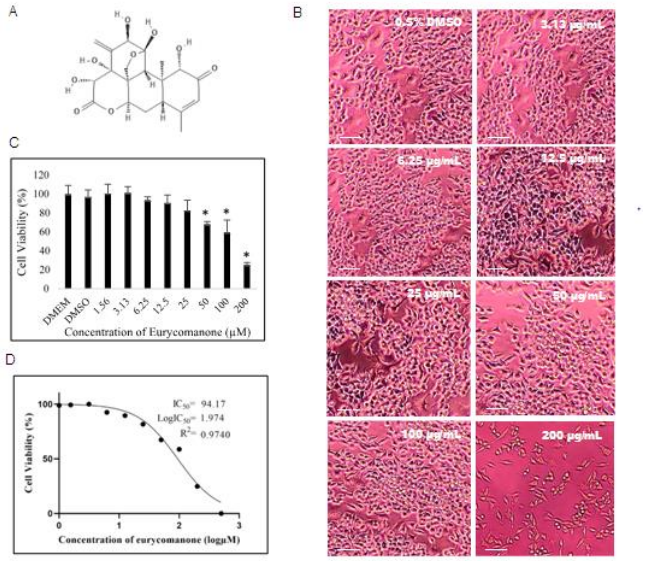

Fig. 1. Cytotoxicity of eurycomanone on RAW 264.7 cells. Briefly, the cells were seeded onto 96-well plate. After overnight, the cells were treated with various concentrations of eurycomanone (1.56-200µM), media and 0.5% DMSO (control) for 24 hours. Then the cells were subjected into MTT Assay. A. The structure of eurycomanone. B. The cell morphology after treatment. C. The cell viability after exposure to eurycomanone. D. Dose-response graph from Graphpad Prism software. IC_{50} of eurycomanone is 94.17µM. *p<0.05, a statistically significant difference from untreated control (0.5% DMSO)

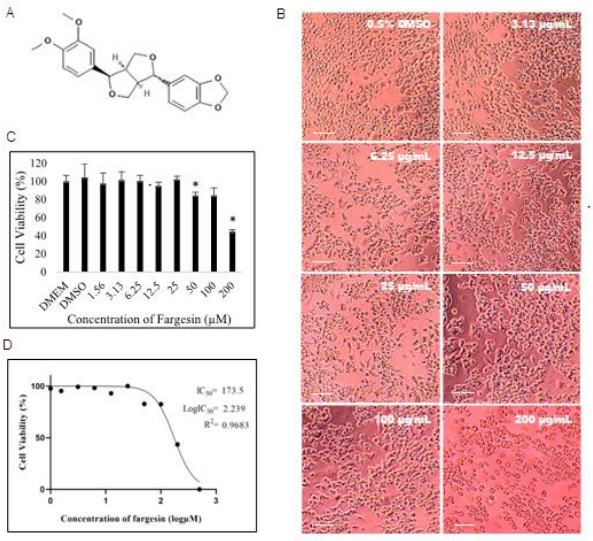

Fig. 2. Cytotoxicity of fargesin on RAW 264.7 cells. Briefly, the cells were seeded onto 96-well plate. After overnight, the cells were treated with various concentrations of fargesin (1.56-200µM), media and 0.5% DMSO (control) for 24 hours. Then the cells were subjected into MTT Assay. A. The structure of fargesin. B. The cell morphology after treatment. C. The cell viability after exposure to fargesin. D. Dose-response graph from Graphpad Prism software. IC_{50} of fargesin is 173.5µM. *p<0.05, a statistically significant difference from untreated control (0.5% DMSO)

used for ages as herbal medicines and previous studies discovered that both of them exhibited anti-inflammatory effect. Eurycomanone and fargesin derived from *Eurycoma longifolia* and *Flosmagnoliae*, respectively are the compound of interest in this current study to discover their anti-inflammatory effect on macrophage RAW264.7 cells. Nevertheless, before further study on the anti-inflammatory effect of these compounds is conducted, it is essential to assess the cytotoxicity of both eurycomanone and fargesin on macrophage RAW264.7 cells.

The potential cytotoxicity of both eurycomanone and fargesin towards macrophage RAW 264.7 cells is evaluated by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. In the MTT assay, the IC_{50} values obtained from the assay indicates the cytotoxicityeffect of the compounds. This assay measures reduction of MTT into insoluble purple formazan in the mitochondria of living cells with the help of mitochondrial succinate dehydrogenase. Reduction of MTT into insoluble purple formazan can only occur in metabolically active cells. The insoluble purple formazan is dissolved with DMSO and spectrophotometer is used to measure the quantity of formazan which is directly proportional to the number of viable cells [26,27].

Most of the cytotoxic studies on eurycomanone derived from *E. longifolia* are about its cytotoxic effect towards cancer cells, which render this compound as a potential candidate for anticancer treatments [7]. In the current study, eurycomanoneexhibitedcytotoxic effect on RAW 264.7 cells with the IC_{50} of 94.7 µM. A study by *Miyake et.al* discovered the cytotoxicity of eurycomanone on four cancer cell lines (colon 26-L5, IC_{50}= >100 µM; B16-BL6 melanoma, IC_{50}= 35 µM; Lewis lung carcinoma (LLC), IC_{50}= >100 µM; human lung A549 adenocarcinomas, IC_{50}= 5.8 µM) [28]. Results from previous studies indicate that eurycomanone showed selective cytotoxicity towards B16-BL6 and A549. The result shows that eurycomanone has a more cytotoxic effect on cancer cells line B16-BL6 and A549 compared to RAW 264.7 cells used in the current study. The difference between the cytotoxic effects might be due to the incubation period of the compound with the cells. Incubation periods of eurycomanone with the cancer cells is 72 hours while RAW 264.7 cells are incubated with eurycomanone for only 24 hours. This result indicates that long-term incubation of the cells with eurycomanone might lead to an increase in cytotoxicity.

Hajjouli et.al discovered that eurycomanone reduced cell viability of K562 and Jurkat human leukaemia cells dose- and time-dependently. Eurycomanone exhibited its cytotoxic effect on K562 and Jurkat leukaemia cells with IC_{50} of 48.92 µM and 40.2 µM, respectively [8]. Similarly, the cell viability of RAW 264.7 cells also been reduced by eurycomanone dose-dependently. However, IC_{50} of eurycomanone (94.7 µM) is higher compared to the IC_{50} of K562 and Jurkat human leukaemia cells which shows that eurycomanone has a less cytotoxic effect on RAW 264.7 cell compared to the human leukaemia cells. A study by *Hajjouli et.al* and current study are conducted at the same incubation period of 24 hours [8].

Additionally, a study by *Wong et.al* highlights that eurycomanone can reduce the cell viability of human lung adenocarcinoma (A549) in a dose-dependent manner at IC_{50} of 5.1 µM [29,30]. Eurycomanone decreased cell viability in a dose-dependent manner are in line with the current study and previous studies conducted by *Wonget.al* and *Zakariaet.al*, on liver cancer cells (HepG2). Eurycomanone reduces the viability of HepG2 cells by 50% inhibition at 3.8±0.12 µM [9,29,30]. Both IC_{50} of eurycomanone on A549 and HepG2 indicate that eurycomanone is more toxic towards these cells compared to macrophages RAW 264.7 cells. The discrepancy in results may be due to the incubation period of the cells with eurycomanone. A549 and HepG2 cells have been incubated for 72 hours while macrophage RAW 264.7 cells are incubated for only 24 hours. These indicate that the incubation period might contribute to the cytotoxic effectsshowed by eurycomanone.

Salahi et.al assessed the cytotoxicity of eurycomanone towards K562 human leukaemia cells and the compound inhibited the viability of K562 cell by 50% at the concentration of 6±1 µM [31]. Eurycomanone is more cytotoxic in K562 cells compared to RAW 264.7 cells in the current study and it could be due to the difference in incubation period between the cells. RAW 264.7 cells have been incubated for only 24 hours while K562 cells for 48 hours. Mahfudh and Hawariah studied the cytotoxic effect of eurycomanone on cancer cells, Coav-3, HeLa, HepG2, HM3KO and MCF-7 and discovered that eurycomanone can decrease the viability of those cancerous cells with IC_{50} of 3.03±0.14 µM,

2.13±0.09 µM, 4.02±0.12 µM, 4.21±0.2 µM and 3.63±0.11 µM, respectively [32]. Eurycomanone is more cytotoxic towards these cancer cells compared to macrophages RAW 264.7 cells**.** Incubation time for cancer cells in the previous study and RAW 264.7 cells in the current study are, 72 hours and 24 hours, respectively. The inconsistency in the result indicates that long-term exposure of the cells to eurycomanone can lead to an increase in cytotoxicity.

Furthermore, a study by *Thu et.al* revealed that root extract of *E. longifolia* did not exert any cytotoxic effect on RAW 264.7 cells upon exposure to different concentration of *E. longifolia* root extract (1, 5, 25, 50 and 100 µM) for 24 hours. The viability of macrophages RAW 264.7 cells in the previous study is more than 95%[33]. The result from the previous study is inconsistent with the current study because, at a concentration of 50 µM, the viability of RAW 264.7 cells is less than 80%. The discrepancy of results might be due to the different type of RAW 264.7 cells used. The current study used RAW 264.7 cell without nitric oxide and the previous study might use parent RAW 264.7 cells. Other than that, current study specifically used eurycomanone which mostly can be found from the root of *Eurycoma longifolia* while the previous study used the root extract of the plant which might have the combination of eurycomanone with other quassinoids.

A study by *Thuet.al* also discovered that long-term exposure to *E. longifolia* might cause intensification in cytotoxicity. Furthermore, a study by *Tran et.al* focus on the anti-inflammatory effect of eurycomanone on TNF-α stimulated HEK-293/NF-κB-luc cells. The inhibition of eurycomanone on NF-κB activity in the cells occur at IC_{50} 2.4 µM [34]. This indicates that eurycomanone is more toxic in inhibiting biological function in TNF-α stimulated HEK-293/NF-κB-luc cells compared to biological function in RAW 264.7 cells.

In the current study, fargesin showed a cytotoxic effect on RAW 264.7 cells with the IC_{50} of 173.5 µM. *Jun et.al* assessed cell viability of human metastatic breast cancer (MDA-MB-231) cells upon treatment with fargesin and viability of MDA-MB-231 cells decrease dose-dependently. This result contradicted with the current study because the viability of RAW 264.7 cells when treated with fargesin do not decrease in a dose-dependent manner. Also, a study by *Junet.al* assess the ability of fargesin to inhibit osteoclasts process in bone marrow macrophages (BBM) dose-dependently with IC_{50} of 4.33 µM [35]. The previous study shows that fargesin is more toxic in inhibiting the biological function of BBM compared to the biological function of macrophage RAW 264.7 cells in the current study. The discrepancy in result between the previous study and current study might be due to different type of macrophage cells used.

A study reported, Fargesin does not exert any toxicity on the viability of THP-1 monocytes cells up to 20 µM[18]. The result is in line with the current study because fargesin does not exhibit any toxic effect on RAW 264.7 cells viability up to the concentration of 20 µM Types of cells used might be the reason for a similar result. The previous study by [18]used the precursor of macrophages, monocytes while the current study used macrophages.

Moreover, a study by *Jiménez-Arellaneset.al* discovered the toxic effect of fargesin on *Entamoeba histolytica* strain HMI-IMSS and *Giardia lamblia* strain IMSS: 0989: 1 with IC_{50} of 120.6 µM and 262.7 µM, respectively, hence render the ability of fargesin as an antiprotozoal [36]. The IC_{50} of fargesin (173.5 µM) on RAW 264.7 cells are lower than the IC_{50} of *Entamoeba histolytica* and *Giardia lamblia*, which indicate that fargesin is more toxic towards RAW 264.7 cells compared to these protozoans. A study by *Kim et.al* assessed the ability of fargesin to inhibit the production of nitric oxide (NO) by BV-2 microglial cells at IC_{50} of 10.4±2.8 µM [15]. This result proves that fargesin is more toxic in inhibiting BV-2 microglial cells biological function compared to biological function in RAW 264.7 cells with the $1C_{50}$ of 173.5 µM [15].

In the current study, IC_{50} of eurycomanone (94.7 µM) is higher than fargesin (173.5 µM), which indicates that eurycomanone has more cytotoxic effect on RAW 264.7 cells compared to fargesin. Based on the dose-response curve of eurycomanone in Figure 1D, the safe dose for eurycomanone that can be used for further pharmacological study on RAW 264.7 cells are between the concentration of 1.56 µM to 12.5 µM. On the other hand, the dose-response curve of fargesin in Figure 2D indicates that the safe dose for fargesin on RAW 264.7 cells is between the concentration of 1.56 µM to 25 µM.

Cytotoxicity of eurycomanone is often associated with its anticancer activity. Eurycomanone exerts its anticancer activity mainly via antiproliferative

effect. Anticancer activity of eurycomanonehas been contributed by the α,β-unsaturated ketone group present in the eurycomanone structure. The α,β-unsaturated ketone group also play a crucial role in anti-inflammatory activity through the inhibition of NF-κB pathway [8,37-40].

The underlying mechanism behind cytotoxicity of eurycomanone and its ability to initiate apoptosis (programmed cell death) is through the upregulation of p53 tumour suppressor protein and pro-apoptotic protein (Bax) with downregulation of anti-apoptotic protein (Bcl-2). Eurycomanone also increases the level of cytochrome C in the cytosol which lead to induction of apoptosis. Eurycomanone is also known to activate caspases, an apoptotic signalling cascade and inhibit NF-κB[29-32,41].

5. CONCLUSION

The results suggested that eurycomanone is cytotoxic towards macrophages RAW 264. 7 cells in a dose-dependent manner. On the contrary, fargesin only exhibits cytotoxicity on RAW 264.7 cells when the concentration is more than 50 μM. Additionally, the IC_{50} of eurycomanone and fargesin are 94.17 μM and 173.5 μM, respectively. The results of this study prove that eurycomanone is more cytotoxic towards macrophages RAW 264.7 cells compared to fargesin. The cytotoxic effect of eurycomanonemight be due to the presence of α,β-unsaturated ketone group in eurycomanone chemical structure. The underlying mechanism for eurycomanone cytotoxicity and its ability to induce apoptosis is might be via the upregulation of pro-apoptotic protein (Bax) and p53 tumour suppressor protein along with the downregulation of anti-apoptotic (Bcl-2). This compound can promoteapoptosis by increasing the level of cytochrome C and active caspases, which is an apoptotic signalling cascade. Sub-lethal dose for eurycomanone and fargesin are 1.56 μM to 25 μM and 1.56 μM to 100 μM, respectively. Even though fargesin is not as cytotoxic as eurycomanone, it is still crucial to determine the sub-lethal dose of both compounds towards RAW 264.7 cells, so that a correct and accurate dose is used for further study to assess the anti-inflammatory effect of these compounds.

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DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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