INVESTIGATE THE CYTOTOXIC AND ANTICANCER PROPERTIES OF METHANOL EXTRACT OF *LEONURUS JAPONICUS*

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ABSTRACTS

*Leonurus japonicus* is a herb commonly used in traditional oriental medicine. Because it is primarily used in the treatment of gynecological diseases, this herb is also known as Motherwort. According to some recent studies, the aqueous extract of *L. japonicus* has the ability to inhibit the growth of some cancer cell lines. We investigate the toxicity of *L. japonicus* methanol extract on several cancer cell lines in this study. The results show that *L. japonicus* methanol extract is potentially effective in leukemia, with an IC50 value of 62.49 ± 9.43 µg/mL on K562 cell lines. Although the extract was toxic to leukemia cell lines, it was not toxic to other cell lines. The IC50 value of BJ and Vero cells at a concentration of 100 g/ml has not been determined.

**Key words:** *Leonurus japonicus*, K562, fibroblast, HCCJ5, MCF-7, Vero, cytotoxic.

I. INTRODUCTION

Cancer is the leading cause of death in the world. In 2020, there will be an estimated 19.3 million new cancer cases (18.1 million excluding non-melanoma skin cancer) and nearly 10.0 million cancer deaths (9.9 million excluding non-melanoma skin cancer) [1]. According to GLOBOCAN 2020, countries with "Low or Medium Human Development Index” will experience the greatest relative increases in cancer incidence by 2040 [2].

For a long time, people have known how to use the available medicinal herbs in nature to prevent and cure diseases. Specifically, some evidence from the Sri Lankan area shows that the Balangoda people knew how to use plants for medicinal purposes 30,000 years ago [3]. According to the following timeline, there is increasing evidence that natural compounds derived from plants are effective in inhibiting the growth of cancer cell lines [4]. More importantly, the use of secondary compounds derived from plants will be safer and fewer side effects for users when compared to chemotherapy [5]. Therefore, the screening of natural compounds derived from plants is also one of the spearhead studies in the modern fight against cancer.

*Leonurus japonicus* also known by the familiar name “Motherwort”, a biennial plant grows wild in many places in the world [6]. Long ago, *L. japonicus* have been present in many traditional remedies in Asia area [7]. Typically in Viet Nam, this herb is also appearance in many traditional medicine remedies for women such as menstrual disorders, dysmenorrhea, menstrual cramps, white discharge, menorrhagia and bleeding [8]. In China, motherwort is essential in the treatment of menstrual and delivery disorders caused by blood stasis, such as dysmenorrhea, amenorrhea and postpartum hemorrhage [7]. Although it has been widely used in traditional medicine, the value of Motherwort in many countries is limited to experience, lacking a lot of scientific evidence. Following the achievements left by the ancients, in-depth studies were born one after another in recent years. Some typical studies have been published such as: vasorelaxant activity [9], coagulant activity [10], angiogenic activity [11], antibacterial activity [12, 13], anti-platelet aggregative activity [14] and an effect on the uterine smooth muscle [15]. Moreover, the cytotoxic activity of this herb has also begun to attract the attention of scientists in the world [16-19].

Therefore, this study was commenced with the objective to evaluate cytotoxic effect of *L. japonicus* on different cancer cell lines which possibly subsidize to the progress of a hopeful anticancer agent.
II. MATERIALS AND METHODS

2.1 Materials

2.1.1. Plant

The dried aerial parts of *L. japonicus* was provided by Thanh Binh pharmaceutical company, Go Vap District, Ho Chi Minh city.

2.1.2. Cell lines

The human leukemia cell lines K562 was received from Prof. Yuko Sato (Tokyo, Japan).

Fibroblast cells, liver cancer (HCC J5) and Vero were provided by Biotechnology Center, Ho Chi Minh city.

Breast cancer cells (MCF7) were provided by the Institute of applied materials science, Vietnam Academy of Science and Technology.

2.2. Methods

2.2.1. Preparation of plant extract

100 gram of dried *L. japonicus* will be ground and soaked in methanol, in such a way that powder is fully cover by solvent. When the powder is fully saturated with methanol, continue to add 200 ml of solvent and shake the obtained at 180 rpm for 24 hours. Filtrate the floating liquid by Whatman filter paper then continue adding 200 ml of new methanol to the remaining residue then repeat those previous steps for 4 times. The flow-through from all extraction will be collected then subjected to rotary evaporator to remove solvent and obtain methanol crude extract [20].

After obtaining the crude extract, dissolve the extract with DMSO solvent to obtain a stock solution with a concentration of 200mg/ml. The extracts were then filtered through 0.45 and 0.22 µm filters, respectively, in a Biological Safety Cabinet. The filtered extract will be refrigerated at -20°C and thawed before use.

2.2.2. Preparation of cells

After being activated, the fibroblast, liver cancer (HCCJ5), leukemia (K562), breast cancer (MCF-7) and Vero cell lines will be loaded into Roux pre-loaded with Roswell Park Memorial Institute 1640 (RPMI 1640) supplemented with 10% FBS (fetal bovine serum) and 5% antibiotics (penicillin/streptomycin), then incubated at 37°C in humidified and CO₂ loaded conditions continuously at 5%.

2.2.3. Cytotoxic assay

The cytotoxic activity of the methanol extract of the plant was determined by the Trypan Blue staining method for leukemia cell lines (K562) [21] and the MTT method [3- (4,5-dimethylthiazol). - 2- yl) - 2,5 - diphenyl tetrazolium bromide] for the remaining adherent cell lines [22]. The methods referenced will be improved to suit the research conditions of the laboratory.

For trypan blue assay, live cells with intact cell membranes are not colored, so have a clear cytoplasm whereas; trypan blue can be absorbed by dead cells, so they have a blue cytoplasm. In each set of experiments, K562 cells and methanol crude extracts are diluted to a suitable concentration range by RPMI medium. Then, the solution containing the cells and diluted extracts will be dissolved in each well of the 6-well plate. In such a way that each well contains 3 ml of K562 cells at a density of 1x10⁵ cells/ml and the extract concentration increases exponentially (6,25 µg/ml; 12,5 µg/ml; 25 µg/ml; 50 µg/ml and 100 µg/ml). The control well consisted of only 3ml of K562 cells at a concentration of 1x10⁵ cells/ml . Shake the plate well and incubate in a CO₂ incubator for 48 hours. 48 h after plating, the medium was transferred to a falcon tube. Attached cells were trypsinized and pooled with cells in tube. This mixture was then briefly centrifuge and suspended in 0.5 ml PBS. A cell suspension was mixed with equal parts trypan blue solution, 0.4% (Merck) and placed in a haematocytometer. Numbers of viable and dead cells were counted separately in two different samples for each plate. Finally by dividing the number of viable cells by the total number of cells, percentage of viable cells was calculated.
For MTT assay, the adherent cell lines were diluted in RPMI medium at a density of 1x10^5 cells/ml. Loaded into 96-well plates, each well 100µl of cell solution. Incubate samples in a CO2 incubator for 24 hours. After 24 h, refill the plate with 100µl extracts of different concentrations and continue to incubate the sample for 48 hours. The concentration in the wells increases exponentially from 3.125 to 200 µg/ml. After treatment with the combined plant extracts, completely remover the old medium and replace it by 200µl of new serum-free RPMI medium, 20µl of filter sterilized MTT (5mg/ml) and incubated at 37°C for 3 hours. The medium with MTT was removed and the formed formazan crystals were solubilized by the addition of 100µl of DMSO and the absorbance read at 570 nm using a universal microplate reader. Treated cells were compared with untreated controls. Tetrazolium salts are cleaved to formazan dye by cellular enzymes only in viable cells. The reduction of the tetrazolium salt MTT, to colored formazan compounds by cellular enzymes only occurs in metabolically active cells (viable cells). Therefore, the amount of formazan dye formed directly correlates to the number of viable cells in the culture and is measured spectrophotometrically as absorbance. Cells exposed to toxins will have decreased activity.

2.2.4. Statistical Analysis

Statistical analysis was done using Graphpad prism. Every experiment was repeated at least 3 times. Results obtained were expressed as means ± SE. The Duncan’s test was used to locate significant differences between means. Significant differences in treatments were accepted at P <0.05.

III. RESULTS

The results of the investigation of the toxic potential of the methanol crude extract of *Leonurus japonicus* against cancer cell lines are shown in detail in Figure 1.

![Figure 1. Cytotoxicity of K562, MCF and J5 of the sample extract (from left to right, respectively)](image)

Through the results in Figure 1, it is clearly that the methanol crude extract of *Leonurus japonicus* is the best effect on the leukemia cancer cell line (K562). In general, the Half Maximal Inhibitory Concentration (IC50) under 100 µg/ml. The mean IC50 value of 3 replicates after statistical processing was 62.49 ± 9.43 (µg/ml). In the investigated concentration range, it can be seen that the extract concentration is inversely proportional to the number of surviving K562 cells.

Unlike leukemia cell lines, both MCF-7 and HCCJ5 cell lines were not inhibited on proliferation at the high concentration range of extracts investigated. Statistical values at 5 concentrations were all different when compared with the control sample, but these differences were not statistically significant. Therefore, it is necessary to increase the concentration of the methanol crude extract to find the IC50 value in the next surveys.

In addition to evaluating the effects of extracts on cancer cell lines, understanding the effects of medicinal herbs on normal cell lines is also an extremely important factor contributing to the decision to conduct research studies preclinical in animal models. Figure 1 is a graph showing the effect of *Leonurus japonicus* methanol crude extract on two normal cell lines (Fibroblast and Vero).
Figure 2. Cytotoxic effects of methanol crude extract on Fibroblast and Vero cells

The results in Figure 2 show that the methanol crude extract of *Leonurus japonicus* has an effect on the proliferation of two target cell lines, the effect of herb is clearly concentration dependent. Fibroblast was slightly more susceptible than Vero cell which might be due to its mortality properties. The criteria of cytotoxicity established by the U.S. National Cancer Institute (NCI) considers a crude extract as active, moderately active, or inactive, when the IC50 values are lower than 20 μg/mL, from 20 to 100 μg/mL, or higher than 100 μg/mL, respectively. Although the concentration range of the survey was relatively large (≥100 μg/ml), the IC50 value of the extract could not be determined. Thus, it can be concluded that the extract is completely safe against the target normal cell lines.

IV. DISCUSSION

In 2003, a research group in New York has published a study on the cytotoxic activity of an aqueous extract of *Leonurus japonicus* on 7 cell lines, including MCF-7. IC50 values obtained ranged from 8 to 40 mg/ml extract after a 48 h incubation period [16]. This is in good agreement with the group's conclusion that the methanol extract of *Leonurus japonicus* belongs to the group with no effect on MCF-7 cells.

In 2009, a research group from the South Africa region has published a paper related to the anticancer activity of *Leonurus japonicus* [17]. The author evaluated the effects of the aqueous extract of *Leonurus japonicus* on HT-29, A549, HL-60, MCF-7 and K562 cells through the MTT method. Again the results showed that the aqueous extract had an effect on cancer cell lines but at a very high concentration, with IC50 values in excess of 500 μg/ml, which is significantly higher than the concentrations that were used in this study. While the aqueous extract of *Leonurus japonicus* is classified as having no potential in inhibiting K562 cancer cells, the methanol extract that our group investigated is classified as a potential group in the treatment of K562 blood cancer.

V. CONCLUSION

Based on the obtained research results, it can be concluded that the methanol extract of *Leonurus japonicus* is a potent inhibitor of K562 leukemia cells and is relatively benign for some normal cell lines constituting animal tissues and organs such as Fibroblast and Vero.

REFERENCES


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