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Foreword

The proceeding is a produced from papers collected during the the Mini Workshop of Southeast Asia Germany Alumni Network (SEAG) on the topic of : "Development of Animal Health and Production for Improving the Sustainability of Livestock Farming in the Integrated Agriculture Systems" held in Bogor-Indonesia on April 25-26th, 2005

Nineteen selected papers were presented in this proceeding from 33 participants which coming from 13 universities in Indonesia and 1 from Thailand.

We would like to highly appreciate and deeply thanks to DAAD for the financial support as a main sponsorship in this Mini Workshop that it made the program very successfully conducted. The same thing is also going to SEAG-Indonesia who fully supported this event as one of their scientific program.

Finally, we would like to thanks to all Steering and Organizing Committee who work very hard for the symposium including the preparation and finalization of this proceeding.

In Vitro Anti-Proliferation and Anti-Invasion Activities of the Combination Between Recombinant Canine Interferon (rCaIFN) with *Luffa cylindrica* Seed Methanol and Chloroform Extracts on MCM-B2 Derived Tumor Cell Line In Collagen Gel Medium

Bambang Pontjo Priosoeryanto[8], Gunanti[9], Hernomoadi Huminto[1] and Ros Sumarny[10]

Abstract

In vitro antiproliferation and anti-invasion activity of the combination between recombinant canine interferon (rCaIFN) and Luffa cylindrica seed methanol and chloroform extracts on MCM-B2 tumor cell lines was studied. The dose of the extract was 100 ppm for the chloroform extract and 350 ppm for the methanol extract, while the dose of rCaIFN was 10^4 IU/ml. The highest anti-proliferation activity of the methanol extract-rCaIFN combination was 84% while the chloroform extract-rCaIFN combination was 60%. The anti-invasion activity was detected on the semi solid medium of collagen gel system. There was an inhibition on the invasion activity of tumor cell to pass the collagen gel on both extract combinations even there was a variation on the inhibition capacity among them. The methanol extract gave the highest inhibition of the cell invasion activity. The ability of the inhibition of cell invasion activity was similar to that of anti-proliferation activity. The result of the present study indicated that the combination of Luffa cylindrica and rCaIFN have a synergetic effect on the inhibition of cell invasion and we concluded that this combination give a promising hope for the tumor disorders treatment. The mechanisms of this combination activity is still unclear and under investigation.

***Key words**: Anti-invasion, anti-proliferation, canine interferon, in vitro, tumor cells line, Luffa cylindrica*

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Introduction

A tumors or neoplasm can be defined as a disturbance of growth characterized by excessive, abnormal and uncontrolled proliferation of transformed or altered tissue at one or more primary points within the host, and frequently at one or more metastatic sites. In the course of spontaneous development of tumor in human and animals, groups of neoplastic cells may be present for years before a tumor. Even after the neoplastic growth becomes detectable, it may remain at relatively stable size and degree of invasiveness for prolonged periods of time before its full malignant potential is manifest.

Interferon (IFNs) has a broad range antiviral immunomodulatory and anti-proliferation effects. In human case, the anti-tumor effects have led to the clinical use of IFN in a variety of diseases (Johnson *et al*, 1994). Recombinant canine interferon (rCaIFN) was produced in a recombinant Baculovirus system by using silkworm (*Bombyx mori*) (Priosoeryanto *et al*, 2000). Anti-proliferation and anti-invasion activities of rCaIFN has been clarified in our previous study (Gunanti *et al*, 2004)

Organics substances isolated from plants are known as metabolite substances. These natural metabolites are widely use in the medical, pharmacy, agro-chemistry and chemical industries (Harborne, 1996). In some Asian countries, metabolites derived from several plants are use for the alternative treatment or traditional medicine for some disorders in human and animals. Our previous studies (Harran *et al*, 2001; Priosoeryanto *et al*, 2001; Tumilisar *et al*, 2001) showed that some plants extracts had an in vitro anti-tumor activitiy by inhibited the tumor cell proliferation.

Indonesia is a tropical country which rich of medicinal plants. Indonesia Drug and Food Control Agency indicated that medicinal herbs were produced in Indonesia by 326 manufacturers and were used not less than 180 medicinal and aromatic plants. The total of raw materials consumption annually reach about 6.223 tons. The Agency was also counted that 45 important drugs in the USA are originated from tropical medicinal and aromatic plants, in fact, 14 plants species are coming from Indonesia. The big number of medicinal and aromatic plants species grow in Indonesia is an indicator that the land and climate conditions of Indonesia very potential for the cultivation development of medicinal and aromatic plants.

The aim of the present study is to elaborate the anti-invasion activity from *Luffa cylindrica* seed methanol and chloroform extracts combined with rCaIFN on the MCM-B2 tumor cell line in vitro, in order to find the potential anti-tumor drugs for medical purposes.

Materials and Methods

Extraction of the Plants

The extracts of *Luffa cylindrica* seed were prepared using methanol and chloroform according to the method of Anonymous, (1985). Briefly, 50 grams each of *Luffa cylindrica* seed powder were macerated using 500 ml of chloroform or methanol and kept for 5 days, and were then filtered. The wastes were dissolved into a sufficient amount of chloroform or methanol and were filtered until the total volume of extracts was 100 ml. The extracts were evaporated to get the desired concentrated filtrates and were kept until use. Working concentrations of each extracts were made by dilution the extracts until the tested concentration was achieved.

Brine Shrimp Lethality Test

Ten larvae of *Artemia salina* on 12 vials each were used (3 concentrations of extracts and one control with 3 replicates). After 24 hours of extracts treatment, the dead *Artemia salina* was counted (Meyer *et al*, 1982). The data were processed statistically using Probit Test.

Anti-proliferation Activity Assay

The MCM-B2 cell lines (Priosoeryanto *et al*, 1995a) were cultured with the density of 10^3 cell/ml on the 24-well dish using a growth medium comprises from DMEM and 10% FCS (Priosoeryanto *et al*, 1995a; 2000). The tested dose of each extracts was determined after the LC_{50} of each extracts were recognized. The extracts were added to the culture dish (3 holes for each dose). For the control positive, anti-tumor commercially drugs Vinblastine was used. After the confluence of cell growth was achieved on the control negative dishes, the cells were harvested and the average of the total number of cells on each dishes were counted using a hemacytometer with Trypan Blue dye. The data were then analyzed to determine the anti-proliferation activity level.

Collagen Gel and Anti-invasion Assay

Collagen type I derived from porcine tendon (Cell matrix IA) was used. The gel was prepared according to the manufacturer recommendation and was then added with DME/F-12 medium, FCS and antibiotic. Collagen gel were stored in petri dishes with 0.3 cm in thick and kept in 37°C, with 5% CO_2 for polimerization. After the gel was polimerized, single tumor-cell solution were added to the surface of the gel. Growth and invasion activities of the tumor cells were observe daily using phase-contrast microscope.

Data Analysis

All quantitative data were statistically analyze, while qualitative data were describe naratively according to Priosoeryanto *et al*, (2000).

Result and Discussion

Brine Shrimp Lethality Test

The LC_{50} for each plant extracts were 66.8287 ppm for chloroform extract and 141,22 ppm for methanol chloroform extract of *Luffa cylindrica*. Based on the LC_{50} we decided to use the dose for anti-proliferation and anti-invasion assays of the extract was 100 ppm for the chloroform extract and 350 ppm for the methanol extract.

Anti-proliferation Activity

The anti-proliferation activity was detected in all extracts combination with rCaIFN. In general, this anti-proliferation activity on the combination form was more higher compared to the extracts or rCaIFN alone. The degree of this activity on both extracts combination was varied. The highest anti-proliferation activity of the methanol extract-rCaIFN combination was 84%, while the chloroform extract-rCaIFN combination was 60% (Figure 1 & 2).

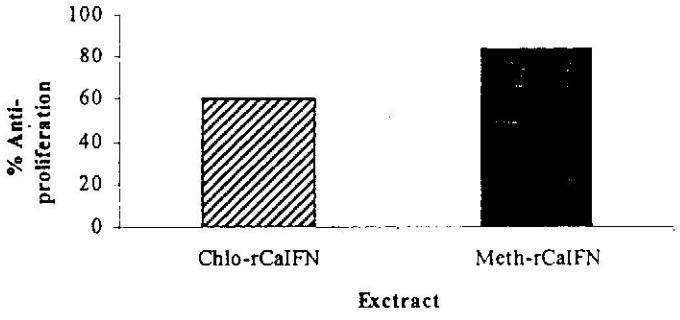

Figure 1. Anti-proliferation activity of combination between rCaIFN-Chloroform and rCaIFN-Methanol extracts of *Luffa cylindica* on MCM B2 cell lines.

There is no report on the activity of combination of IFN with plant extracts in order to treat tumor dissorder, even activity of the combination between IFN with comercially anti-tumor drugs has been reported before. Several inverstigator indicated that there was an increasing of anti-tumor activity on the combination between IFN with some anti-tumor substances such as Decarbazine, Vincristin, Bleomycin dan Lomustine (Pyrhoenen *et al,* 1992), Fluorouracil (Raderer and