Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines

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ARTICLE INFO

Article history:
Received 1 March 2012
Received in revised form 24 March 2012
Accepted 28 April 2012
Available online 28 October 2012

Keywords:
Cytotoxicity
Growth inhibitory
Elaeis guineensis
Cell morphology

ABSTRACT

Objective: To investigate the cytotoxic effect of *Elaeis guineensis* methanol extract on MCF-7 and Vero cell. **Methods:** *In vitro* cytotoxicity was evaluated in by MTT assay. Cell morphological changes were observed by using light microscope. **Results:** The MTT assay indicated that methanol extract of the plant exhibited significant cytotoxic effects on MCF-7. Morphological alteration of the cell lines after exposure with Elaeis guineensis extract were observed under phase contrast microscope in the dose dependent manner. **Conclusions:** The results suggest the probable use of the *Elaeis guineensis* methanol extract in preparing recipes for cancer-related ailments. Further studies on isolation of metabolites and their *in vivo* cytotoxicity are under investigation.

1. Introduction

The crescendo of a new anticancer agent begets in company with new leading bioactive compounds being identified. Normatively, new bioactive compounds displaying anticancer activities are obtained from pharmaceutical industries and research laboratories that relativize to academic institutions. Major resources scilicet natural products and synthetic compounds analog to known agents have been disclose in possessing various bioactive compounds yearned by the drug development industries. The evaluation and the discovery of new anticancer agents is long-term process that encompasses many steps. The step broaches with the screening for anticancer properties, followed by the isolation and identification of bioactive compounds obliged to anticancer properties, toxicity estimation of the isolated compounds and finally in vivo anticancer activity testing to verify the aptitude of the compounds. The winnowing natural products particularly plant extracts after effect the breakthrough of few excellent anticancer agents. The vinca alkaloids (vincristine, vinblastine and vindesine) and the podophylotoxin derivatives (etoposide and teniposide) are examples of clinically active plant products[1]. The goal of screening medicinal plant is to search for excellent anticancer agent avertable to human malignancies. In defiance of astonishing advances in modern medicine, such as surgery, radiotherapy, chemotherapy, and hormone therapy, cancer disease remains a worldwide health problem, thus endeavoring the search for new alternate approach. The nature as a huge valuable contributor of potential source for chemotherapeutic agents has recently been reviewed[2]. Newman and Cragg[2] reported in their analysis that the sources of new drugs over the period 01/1981-06/2006 possess 974 small molecules, out of which 66% were new chemical entities which are classified synthetic, 17% correspond to synthetic molecules containing pharmacophores derived directly from natural products, and 12% are actually modeled on a natural product inhibitor of the molecular target of interest, or mimic (i.e., competitively inhibit) the endogenous substrate of the active site, such as ATP. These facts are in favor with the new call for medicinal plant identification namely local plants, in conjunction with anticancer properties. Since the methanol extract of *Elaeis guineensis* (*E. guineensis*) insinuated good biological activity earlier, considerably evaluation on the anticancer potentiality is discussed in this study. The current study was undertaken with the objective to rationalize the cytotoxicity effect of *E. guineensis* methanol extract on MCF-7 and Vero cell lines in accordance to the observable changes of cell

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Foundation project: This work was partly supported by USM Incentive Grant (Grant Number: 2009/167) from Universiti Sains Malaysia.

morphology upon exposure to the extract.

2. Materials and methods

2.1. Plant material and extraction

The leaves of *E. guineensis* were collected in Semeling, Kedah, Malaysia around January 2010. Plant material was air dried in the laboratory for 5 days at room temperature followed by oven drying at 40oC then grinded to powder form using an electric mill. The powdered sample was kept in an air tight container until required. About 45 g of the powdered leaves of *E. guineensis* was macerated in 250 mL of methanol for 72 h. Rotary evaporator was used to filter and concentrated methanolic plant material at 40oC and the resulting extract was kept in the refrigerator.

2.2. Cytotoxicity Screening

2.2.1. Cell Lines

All cell lines used during the present study were obtained from Tissue Culture Laboratory of Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Pulau Pinang, Malaysia.

The Vero cell line was initiated from kidney of a normal adult African green monkey on March 27th, 1962, by Yasummura and Kawakita at the Chiba University, Japan American Public Health Association, 1992). Vero cells were maintained in RPMI-1640 medium supplemented with 10% FBS, glutamine (2 raM), penicillin (100 units/mL) and streptomycin (100 μg/mL). The cells were cultured at 37℃ in a humidified 5% CO_2 incubator.

Human breast adenocarcinoma (MFC-7) cells were derived from breast cancer which was obtained from American Type Culture Collection (ATCC: Manassas, VA). MCF-7 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, glutamine (2 raM), penicillin (100 units/mL) and streptomycin (100 μg/mL). The cells were cultured at 37℃ in a humidified 5% CO_2 incubator.

2.1.2 Cytotoxicity assay

The extract of *E. guineensis* leaf was tested for *in vitro* cytotoxicity, using Vero and MCF-7 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[3]. Briefly, 100 μL of media (RMPI 1640) was added into each of the 96-well plates from row B to row G (triplicate). Then, 100 μL of diluted plant extract or fractions were added in row A and row *B. Starting* from row B the 200 μL of solution (100 μL drug + 100 μL media) were mixed and 100 μl from row B were added into next row (row C) by using micropipette and a serial dilution was done up to row G. Finally, excessive 100 μL from row G were discarded. The final volume for each well was 100 μL. The cultured Vero/ MCF-7 cells were harvested by trypsinization, pooled in a 50 mL vial. Then, the cells were plated at a density of 1 ×106 cells/mL cells/well (100 μL) into 96-well micro-titer plates from row B to row G. Finaly, 200 μL of cells (Vero/ MCF-7) were added in row H as a control. Each sample was replicated 3 times and the cells were incubated at 37 ℃ in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 μL of 5 mg/mL) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under a microscope. Flowingly, the medium together with MTT (190 μL) were aspirated off the wells, DMSO (100 μL) was added and the plates shaken for 5 min. The absorbance for each well was measured at 540 nm in a micro-titre plate reader[3] and the percentage cell viability (CV) was calculated manually using the formula:

$$CV = \frac{\text{Average abs of duplicate drug wells}}{\text{Average abs of control wells}} \times 100\%$$

A dose-response curve were plotted to enable the calculation of the concentrations that kill 50% of the Vero/ MCF-7 cells (IC_{50}).

2.2.3 Morphological analysis

Morphological observation of cell treated with *E. guineensis* extract from cytotoxicity study was done to determine the changes induced by the extracts. Changes such as shrinking of the cells, membrane blebbing, ballooning, chromatin condensation, formation of apoptotic bodies were observed in predicting the apoptotic mechanism for cell death. Meanwhile, vacuolations of the cytoplasm and formation of double membrane vesicle containing organelles were assessed for authophagic cell death.

3. Results

3.1. Proliferative effects of MCF-7 and Vero cells

The effect of anticancer from *E. guineensis* on MCF-7 and Vero cell lines was evaluated thorugh micro-culture tetrazolium assay (MTT). The multiple concentrations of methanolic extract from *E. guineensis* were used and effective doses were calculated from dose-response curve. Results of the cytotoxicity evaluation against MCF-7 and Vero cell line of the *E. guineensis* extract are shown in Figure 1 and 2. The methanol extract of *E. guineensis* exhibited no significant activity against the Vero cell line achieving an IC_{50} value of 22.00 μg/mL. On the contrary, the methanol extract of *E. guineensis* exhibited significant activity against the MCF-7 cell line with an IC_{50} value of 15.00 μg/mL. The criteria of cytotoxicity for the crude extract, as established by the U.S. National Cancer Institute (NCI), is an $IC_{50} < 20$ μg /mL in the preliminary assay[4]. On treatment with *E. guineensis* extract, the MCF-7 cells showed an increased rate of cell death at a lower concentration of the extract when compared to that in the Vero cells (Figure 1 and 2).

3.2. Evaluation on morphological changes upon treatment with extracts

Morphological alteration of MCF-7 and Vero cells lines upon exposure using *E. guineensis* extract was observed under phase contrast microscope. The cells indicated the most prominent effects after exposure to the *E. guineensis*