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Comparing the Cytotoxicity Properties and Mechanism of *Lawsonia inermis* and *Strobilanthes crispus* Extract Against Several Cancer Cell Lines

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This research was conducted to study and compare the cytotoxic effect of the extracts of two plants, henna (*Lawsonia inermis*) and kejibeleng (*Strobilanthes crispus*) on several kinds of cancer cell lines. The mechanism of the effect was also studied through the expression of cancer-caused gene, *c-myc*. This research was done *in vitro* using several kinds of cancer cell lines such as human colon cancer cell lines (Caco-2), liver cancer cell lines (HepG2), hormone-dependent breast cancer cell lines (MCF-7) and hormone-independent breast cancer cell lines (MDA-MB-231) and Chang Liver cell lines. The cytotoxic effect was measured through MTT assay and the potential cytotoxic value was calculated by determining the toxic concentration which may kill up to 50\% of the total cell used (IC\textsubscript{50}). Meanwhile, the cytotoxic mechanism was studied by determining the effect of adding both extracts to the *c-myc* gene expression. The methods for determination were RT-PCR and sequencing process. The results showed that chloroform extract from henna can be used against human colon cancer cell lines (Caco-2) and liver cancer cell lines (HepG2) with an IC\textsubscript{50}-value of 25.1 and 28 µg mL\textsuperscript{-1}, respectively. Kejibeleng was also found to be cytotoxic against human liver cancer cell lines (HepG2) and hormone-dependent breast cancer cell lines (MCF-7) with an IC\textsubscript{50}-value of 0.3 and 24.8 µg mL\textsuperscript{-1}, respectively. However, the extract was not cytotoxic against human colon cancer cell lines (Caco-2). The smallest value of IC\textsubscript{50} was seen when the kejibeleng extract was compared with henna. The cytotoxic effect of both plant extracts may be mediated by the down-regulation of *c-myc* expression.

**Key words:** C-myc gene expression, RT-PCR, cancer cell lines, anticancer agents
INTRODUCTION

Henna (*Lawsonia inermis* Linn) is a plant, which grow wild in abandoned areas (Muhammad and Mustafa, 1994) and commonly known as 'mai' in Sumatra or 'Pachar kuku' in Java. This plant is a worldwide known cosmetic agent used to color hair, skin and nails (Hanna et al., 1998). However, it is not only relevant to cosmetics. Henna was also reported to have tuberculostatic activity (Sharma, 1990). The leaves are also used as a prophylactic against skin diseases. They are used externally in the form of paste or decoction against boils, burns, bruises and skin inflammations. A decoction is used as a gargle against sore throat (Rout et al., 2001). The roots of this plant are useful in burning sensation, leprosy, strangury and premature greying of hair (Vaidyaratnam, 1995).

The major phytochemical constituents of henna, lawsone, is found to possess significant anti-inflammatory, analgesic and antipyretic activities (Ali et al., 1995). Recently, this compound has been reported to have a growth inhibitory effect against human colon carcinoma, HCT-15 cells (Kamei et al., 1998).

*Strobilanthes crispus* ZII 109 (L) Brenek or *Saricocalix crispus* ZII 109 (L) Brenek (Acanthaceae) plant is a native to countries from Madagascar to Indonesia (Sumarto, 1977) and was first quoted by Anderson, Thomas who classified the plant under Spermaphyta (Flowering plants and Gymnosperma) (Brummitt and Powell, 1992).

A study in Indonesia found that an infusion of the dried leaves of *S. crispus* has been used as antidiabetic, diuretic, antilicytic and laxative agents. A recent study indicated that the water extract of *S. crispus* contained compounds with very high binding affinity to protein molecules that bind the active part of reverse transcriptase. It inhibits the proliferation of retrovirus, an agent in viral disease such as acquired immune deficiency syndrome (AIDS) and Adult T-cell Leukemia (Kusumoto et al., 1992).

The present study aimed at comparing two different plants, *Lawsonia inermis* and *Strobilanthes crispus* with regard to their cytotoxic and mechanism activities in several kinds of human cancer cell lines.

MATERIALS AND METHODS

Cytotoxicity studies

Plant materials and extractions: The research work was done at Department of Nutrition and Health Sciences, Universiti Putra Malaysia from June 2002 until June 2003. The leaves of *L. inermis* and *S. crispus* were harvested at the Faculty of Medicine and Health Sciences, UPM, Serdang, Selangor. The herbarium voucher specimen were identified and deposited by Mr. Ahmed Zainuddin from the Department of Botany, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The voucher number of *L. inermis* and *S. crispus* were AZ-6804 and AZ-6803, respectively.

The extraction methods were obtained from Ali et al. (1996) with slight modification.

Culturing of cells: HepG-2, Caco-2, MDA-MB-231, MCF-7 and Chang Liver cell lines were obtained from American Type Culture Collection (ATCC, USA). The medium for HepG-2 and Chang liver were Minimum Essential Medium with Earle's salt (Gibco, USA). While Caco-2, MDA-MB-231 and MCF-7 were grown by using Dulbecco's Modified Eagle medium (Gibco, USA). The cells were cultured in their own medium supplemented with 10% of fetal calf serum, 100 IU mL⁻¹ penicillin and 100 μg mL⁻¹ of streptomycin (Gibco, USA) using 25 cm² flasks (Nunc, Denmark) in a CO₂ incubator (Sanyo, Japan) at 37°C.

MTT assay: Cytotoxicity effect was determined by the tetrazolium salt method (MTT method), according to the manufacturer's instructions (Roche Diagnostic, USA).

Study of the mechanism

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR): The isolation of mRNA was performed using the Micro-FastTrack™ 2.0 kit (Invitrogen, USA). The RT-PCR process was carried out by using cDNA Cycle kit (Invitrogen, USA). The polymerase Chain Reactions were performed by 30 cycles amplification for 1 min at 94°C, 2 min at 55°C and for 3 min at 72°C. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel.

The sequences of primers were as follows:

- c-myc sense: 5'-CAAGAGGGCAAGACAAACGTCT-3'
- c-myc antisense: 5'-AACTTGTTCGTCTATTTCCGCAA-3'

Sequencing: The sequencing technique was done by using an Automatic Sequencer (USA) and the chromatograms were analysed by using the Chromatos software. The similarities in the sequences of samples were compared with the sequence in the database of genes using the BLAST program.

RESULTS

The chloroform extract of *S. crispus* has shown to be cytotoxic against Caco-2 (IC₅₀=25.1 μg mL⁻¹) and human liver cancer cell lines, HepG2 (IC₅₀ = 28 μg mL⁻¹) (Fig. 1).
Fig. 1: The effect of *S. crispus* on different human cell lines. Cells were plated with $1 \times 10^6$ cells per well in 96-well culture plates. After 72 h incubation at 37°C, MTT assay was applied to assess cytotoxic effect. IC$_{50}$ of 25.1 and 28 µg mL$^{-1}$ were obtained against Caco-2 and HepG2, respectively.

Fig. 2: The effect of chloroform extract of *L. inermis* on different human cell lines. The protocol applied was similar to the previous one. IC$_{50}$ of 0.3 and 24.8 µg mL$^{-1}$ were obtained against HepG2 and MCF-7, respectively (Susi Endrini *et al.*, 2002).

On the other hand, the chloroform extract of *L. inermis* has shown to be very cytotoxic to human liver cancer cell line (HepG2) and also toward hormone-dependent breast cancer cell line (MCF-7) with IC$_{50}$ value of 0.3 and 24.8 µg mL$^{-1}$, respectively. No cytotoxic effect was detected in Caco-2 (Fig. 2).

The results showed that the c-myc genes (218 bp) were expressed in untreated HepG2 and Caco-2 cell lines (Fig. 3 and 4).

In contrast, Fig. 3 showed that the c-myc genes were not expressed in HepG2 cells treated with 20 and 30 µg mL$^{-1}$ *L. inermis* crude extract. The c-myc gene was also not expressed in HepG2 cell treated with 30 µg mL$^{-1}$ *S. crispus* crude extract. However, the c-myc expression was still observed in HepG2 cells treated with a low dose (20 µg mL$^{-1}$) of this extract (Fig. 4). The similar pattern were also observed in Caco-2 cell treated with this extract (Fig. 5).
Fig. 5: Effect of S. crispus crude extract on the expression of c-myc gene in Caco-2 cell line. PCR products were analysed on a 1.5% agarose gel. M, 100 bp DNA ladder marker; lane 1, Caco-2 control (untreated); lane 2, Caco-2 treated with 20 μg mL⁻¹ S. crispus crude; lane 3, Caco-2 treated with 30 μg mL⁻¹ S. crispus crude. c-myc gene at lane 1 and 2 was 218 bp in length. The gene expression was not observed in lane 3.

The confirmation of c-myc gene was performed by using sequencing technique and the percent similarity was achieved by using BLAST software from gene bank database. In this study, the 218 bp DNA ladder was confirmed as human c-myc gene with 91% similarity.

DISCUSSION

The chloroform extract of henna displayed the strongest cytotoxic effect on human liver cancer cell line (HepG2) with an IC₅₀ value of 0.3 μg mL⁻¹. On the other hand, the chloroform extract of S. crispus has shown to be cytotoxic against Caco-2 and HepG2 with an IC₅₀ value of 25.1 and 28 μg mL⁻¹, respectively. According to Wall et al. (1987) any plant extracts with an IC₅₀-value below 20 μg mL⁻¹ can be accepted as a potent cytotoxic extract.

To observe the effectiveness of henna and S. crispus crude extracts in suppressing oncogenes, mRNAs were extracted from the treated cells. Due to instability of RNA and for the PCR purpose, mRNA was converted to cDNA before proceeding to the PCR process. The PCR has been selected as the most suitable technique to amplify the quantity of oncogenes, so that the suppression of oncogenes can be visualized clearly after a gel electrophoresis analysis. Besides, different oncogenes have their own temperature for denaturation, annealing, elongation in a number of cycles to get the best PCR products.

Expression of the nuclear proto-oncogenes, c-myc is indicative of early response during cell proliferation and it has been found to be frequently overexpressed in a variety of tissues and cultured cancer cell lines (Saito et al., 1991). Many investigators have found that down-regulation of c-myc expression may be mandatory for the induction of apoptosis in leukemia cells (Alnemri et al., 1992), macrophages cells (Oritani et al., 1992), prostate cancer (Balaji et al., 1997) and lung cancer (Van Waardenburg et al., 1997).

Many plant extracts have been reported to inhibit cell proliferation through the down regulation of c-myc expression (Jiang et al., 2006; Woo and Choi, 2005). In this study, the c-myc expression was suppressed by crude extract from both plants. The effect depended on the doses given and it seemed to be correlated with the IC₅₀ value of each treatment.

CONCLUSION

This present study verifies that L. inermis was most cytotoxic against human liver carcinoma cell lines, HepG2. On the other hand, S. crispus was shown to be more cytotoxic against colon carcinoma cell lines, Caco-2 than L. inermis. The cytotoxic effect of both plant extracts may be mediated by the down-regulation of c-myc expression.

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