Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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J. Med. Sci., 7 (7): 1098-1102
1st October, 2007

Comparing of 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 kejibeling (*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_{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_{50}-value of 25.1 and 28 μg mL^{-1}, respectively. Kejibeling 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_{50}-value of 0.3 and 24.8 μg mL^{-1}, respectively. However, the extract was not cytotoxic against human colon cancer cell lines (Caco-2). The smallest value of IC_{50} was seen when the kejibeling 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

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INTRODUCTION

Henna (*Lawsonia inermis* Linn) is a plant, which grow wild in abandoned areas (Muhammad and Mustafa, 1994) and commonly known as 'inai' in Sumatra or 'Pachar kuku' in Java. This plant is a worldwide known cosmestic 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 gurgle 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 antiinflammatory, 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) Bremek or *Saricocalix crispus* ZII 109 (L) Bremek (Acanthaceae) plant is a native to countries from Madagascar to Indonesia (Sunarto, 1977) and was first quoted by Anderson, Thomas who classified the plant under Spermatophyta (Flowering plants and Gymnosperma) (Brummit and Powell, 1992).

A study in Indonesia found that an infusion of the dried leaves of *S.crispus* has been used as antidiabetic, diuretic, antilytic 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 $\mu g\ mL^{-1}$ of streptomycin (Gibco, USA) using 25 cm^2 flasks (Nunc, Denmark) in a CO_2 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 by 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'-CAAGAGGCGAAGACACAACGTCT-3'
c-myc antisense : 5'-AACTGTTCTCGTCGTTTCCGCAA-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 (IC_{50}=25.1 $\mu g\ mL^{-1}$) and human liver cancer cell lines, HepG2 (IC_{50} = 28 $\mu g\ mL^{-1}$) (Fig. 1).

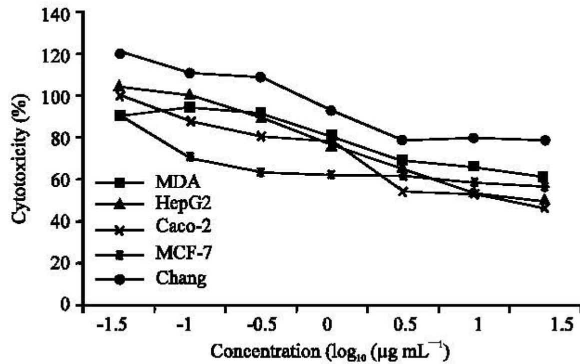

Fig. 1: The effect of *S. crispus* on different human cell lines. Cells were plated with 1×10^5 cells per well in 96-well culture plates. After 72 h incubation at 37°C, MTT assay was applied to assess cytotoxic effect. IC_{50} of 25.1 and 28 μg mL^{-1} were obtained against Caco-2 and HepG2, respectively

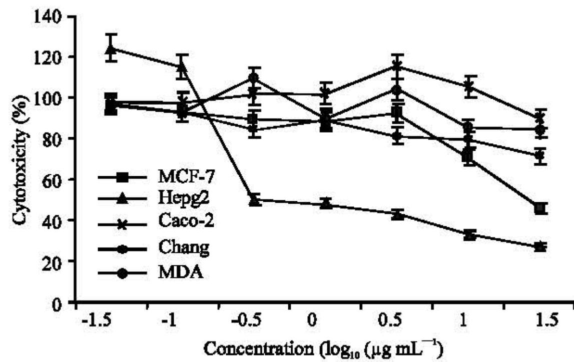

Fig. 2: The effect of chloroform extract of *L. inermis* on different human cell lines. The protocol applied was similar to the previous one. IC_{50} of 0.3 and 24.8 μg mL^{-1} were obtained against HepG2 and MCF-7, respectively (Susi Endrini *et al*., 2002)

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).

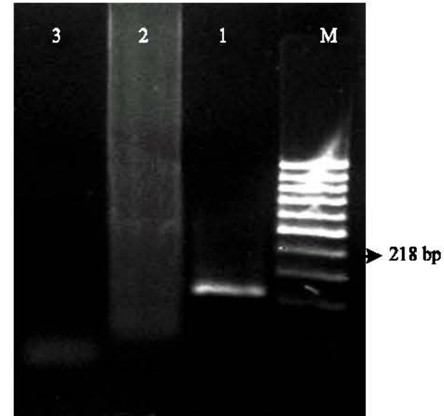

Fig. 3: Effect of *L. inermis* crude extract on the expression of *c-myc* gene in HepG2 cell line. PCR products were analysed on a 1.5% agarose gel. M, 100 bp DNA ladder marker; lane 1, HepG2 control (untreated); lane 2, HepG2 treated with 20 μg mL^{-1} *L. inermis* extract; lane 3, HepG2 treated with 30 μg mL^{-1} *L. inermis* extract. *C-myc* gene at lane 1 was 218 bp in length. The gene expression was not observed in lane 2 and 3

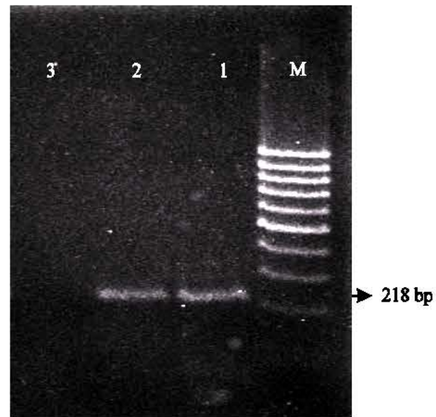

Fig. 4: Effect of *S. crispus* crude extract on the expression of *c-myc* gene in HepG2 cell line. PCR products were analysed on a 1.5% agarose gel. M, 100 bp DNA ladder marker; lane 1, HepG2 control (untreated); lane 2, HepG2 treated with 20 μg mL^{-1} *S. crispus* extract; lane 3, HepG2 treated with 30 μg mL^{-1} *S. crispus* extract. *C-myc* genes at lane 1 and 2 was 218 bp in length. The gene expression was not observed in lane 3.

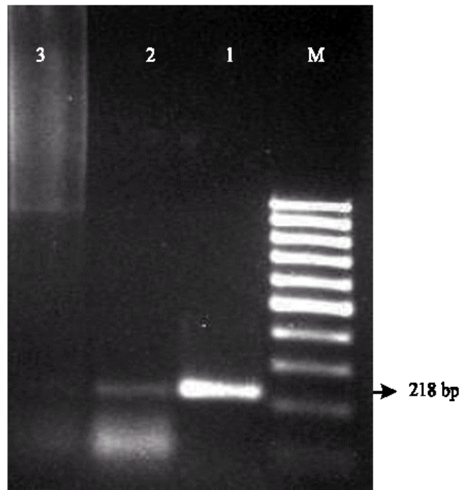

Fig. 5: Effect of *S. crispus* crude extract on the expression of *c-myc* gene in Caco-2 cell line. PCR products were analysed on a 1.5% agarose gel. M, 100 bp DNA ladder marker; lane 1, Caco-2 control (untreated); lane 2, Caco-2 treated with 20 µg mL^{-1} *S. crispus* crude; lane 3, Caco-2 treated with 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 choloroform extract of henna displayed the strongest cytotoxic effect on human liver cancer cell line (HepG2) with an IC_{50} 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 IC_{50} value of 25.1 and 28 µg mL^{-1}, respectively. According to Wall *et al.* (1987) any plant extracts with an IC_{50}-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 IC_{50} value of each treatment.

CONCLUSION

This present study verifies that *L. inermis* was most cytotoxic against human liver carcinoma cell lines, HepG-2. 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.

ACKNOWLEDGMENT

The authors thank IRPA grant 06-02-04-0050.

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