ANTIOXIDANT ELEMENTS ANALYSIS AND ENZYME ACTIVITIES IN
HEPATOCARCINOGENESIS INDUCED RATS TREATED WITH
STROBILANTHES CRISPUS

By

YOGESPIRIYA A/P SHREERAMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
In Fulfilment of the Requirements for the Degree of Master of Science

November 2004
Specially dedicated to

My lovely parents,

MR. SHREERAMAN A/L RAMASAMY and
MRS. LALITHA A/P RAJAMANIKAM

My husband,

MR. SURESH A/L PALPANABAN

My brothers, sister in law and their daughter

MR. PUNITHAN
MRS. ALAMELU MANGAI
MR. AMITEN
MISS SRI SHAANKEREY
ANTIOXIDANT ELEMENTS ANALYSIS AND ENZYME ACTIVITIES IN HEPATOCARCINOGENESIS INDUCED RATS TREATED WITH *STROBILANTHES CRISPUS*

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YOGESPIRIYA A/P SHREERAMAN

November 2004

Chairman: Associate Professor Fauziah Othman, Ph.D

Faculty: Medicine and Health Sciences

This study was conducted to investigate the effect of 5% (w/v) *Strobilanthes crispus* crude extract on rat liver during induced hepatocarcinogenesis. Elemental analysis, was done to support this study on all the liver tissues by energy filter transmission electron microscope (EFTEM) and variable pressure scanning electron microscope (VPSEM). Histological and gross changes of liver evaluation were conducted to observe the cellular and morphological changes during induced hepatocarcinogenesis in rats treated with *S.crispus*. Xenobiotics detoxification enzymes such as aniline hydroxylase (ANH) and glutathione S-transferase (GST) were assayed in liver tissues. Gross examination on liver of rats which were induced with diethyl nitrosamine/acetylaminofluorene (DEN/AAF) showed the presence of nodules, haemorrhages and swelling on liver surface. The 5% (w/v) *Strobilanthes crispus* extract effectively reduced gross changes on liver during induced hepatocarcinogenesis. Meanwhile, histological evaluation revealed that a certain grade of inflammation or necrosis at portal and lobular region and stages of fibrosis
during induced hepatocarcinogenesis was successfully reduced after the administration of *Strobilanthes crispus* extract. Nevertheless, supplementation of *S. crispus* did not fully recover the liver to normal histological feature. This could be due to the short duration of this experiment. Elemental analysis by VPSEM showed that 5% w/v *Strobilanthes crispus* extract contained 63.52% of carbon, 16.56% of oxygen, 0.66% of sodium, 0.08% of magnesium, 11.78% of aluminium, 0.17% of phosphorus, 1.19% of sulphur, 1.66% of chloride, 0.95% of potassium, 0.19% of iron, 0.20% of copper, 2.30% of calcium, 0.39% silicon and 0.35% of argentum. The fresh liver tissue obtained from rats administered with DEN/AAF and treated with *Strobilanthes crispus* extract showed higher percentage distribution of antioxidant elements such as potassium and magnesium when compared to the liver from rats induced with DEN/AAF and untreated rats, DEN/AAF administered and treated with glycyrrhizin rats, normal without treatment rats, normal with *Strobilanthes crispus* supplemented rats and normal with glycyrrhizin supplemented rats. To detect the antioxidant elements such as potassium, calcium, magnesium and iron at ultrastructural level, EFTEM was used. By utilizing EFTEM, the distributions of these elements were also found to be higher in cancer with *S.crispus* group compared to other groups. This may suggest showed that tumor cells has high uptake of these elements from *S.crispus* extract. Meanwhile, DEN/AAF induced rats showed an increase activity of drug/carcinogen detoxification enzymes i.e. GST and ANH. However, 5% w/v *S.crispus* extract effectively inhibit the activity of these enzymes in DEN/AAF induced rats. *S.crispus* which is rich with antioxidant elements such as potassium, magnesium, calcium and iron play important role during carcinogenesis. Thus, *S.crispus* can be considered as potential chemopreventive agent
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS ELEMEN-ELEMEN ANTIOKSIDAN DAN AKTIVITI ENZIM SEMASA KARSIINOGENESIS HEPAR DIARUH DALAM TIKUS DIRAWAT DENGAN STROBILANTHES CRISPUS

Oleh

YOGESPIRIYA A/P SHREERAMAN

Mei 2004

Pengerusi: Profesor Madya Fauziah Othman, Ph.D

Fakulti: Perubatan dan Sains Kesihatan

lobular hepar dan peringkat fibrosis semasa karsinogenesis hepar diaruh berjaya
dikurangkan setelah diberi 5% ekstrak *Strobilanthes crispus* kepada tikus.
Walaubagaimanapun, *Strobilanthes crispus* tidak dapat merawat perubahan ini ke tahap
normal. Ini mungkin disebabkan tempoh eksperimen yang singkat. Elemen-elemen yang
dianalisis dengan VPSEM menunjukkan bahawa 5% ekstrak *Strobilanthes crispus* terdiri
daripada 63.52% karbon, 16.56% oksigen, 0.66% natrium, 0.08% magnesium, 11.78%
aluminium, 0.17% fosforus, 1.19% sulfur, 1.66% klorin, 0.95% kalium, 0.19% besi,
0.20% kuprum, 2.3% kalsium, 0.39% silikon dan 0.35% argentum. Tisu hepar segar dari
kumpulan tikus yang disuntik dengan DEN/AAF dan dirawat dengan *Strobilanthes
crispus* menunjukkan peratus taburan elemen-elemen antioksidan seperti kalium dan
magnesium yang tinggi jika dibandingkan dengan tisu hepar daripada tikus yang disuntik
dengan DEN/AAF yang tidak dirawat, tikus yang disuntik dengan DEN/AAF dan dirawat
dengan gliserizin, tikus normal, tikus normal yang diberi *Strobilanthes crispus* dan tikus
normal yang diberi gliserizin. Bagi menganalisis elemen-elemen antioksidan seperti
kalium, kalsium, magnesium dan besi pada peringkat ultra struktur, EFTEM digunakan.
Menurut EFTEM, taburan elemen-elemen ini didapat paling banyak dalam kumpulan
kanser dengan rawatan *Strobilanthes crispus* jika dibandingkan dengan kumpulan-
kumpulan lain. Ini menunjukkan sel-sel tumor mengambil elemen-elemen tersebut dari
ekstrak *Strobilanthes crispus* untuk memenuhi keperluannya. Sementara itu, suntikan
DEN/AAF ke atas tikus menunjukkan peningkatan aktiviti enzim yang menyakitkan
drug atau karsinogen seperti GST dan ANH pada hepar. Walaubagaimanapun, 5%
*Strobilanthes crispus* dapat merencat aktiviti enzim-enzim ini dengan efektif pada tikus
yang disuntik dengan DEN/AAF. *Strobilanthes crispus* adalah kaya dengan elemen-
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I certify that an Examination Committee met on 3rd November 2004 to conduct the final examination of Yogespiriya A/P Shreeraman on her Master of Science thesis entitled “Antioxidant Elements Analysis and Enzyme Activities in Hepatocarcinogenesis Induced Rats Treated with Strobilanthes crispus” in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the supervisory committee are as follows:

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Date:
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

YOGESPIRIYA D/O SHREERAMAN

Date:
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<td>HCC</td>
<td>hepatocellular carcinoma</td>
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<td>DEN</td>
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<td>AAF</td>
<td>acetylaminofluorene</td>
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<td>PCD</td>
<td>programmed cell death</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>DMBA</td>
<td>7,12-di-methylbenz[a]anthracene</td>
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<td>EGCG</td>
<td>epigallocatechin gallate</td>
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<td>AFB</td>
<td>aflatoxin B1</td>
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<td>TLC</td>
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<td>ROS</td>
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<td>BHA</td>
<td>butylated hydroxyanisole</td>
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<td>BHT</td>
<td>butylated hydroxytoluene</td>
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<td>ATP</td>
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<td>NF</td>
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<td>rpm</td>
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<td>DNA</td>
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<td>transmission electron microscope</td>
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<td>energy filter transmission electron microscope</td>
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<td>variable pressure scanning electron microscope</td>
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<td>RDA</td>
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<td>-OH</td>
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CHAPTER 1

INTRODUCTION

Around 10 million new cancer patients are diagnosed worldwide each year and these rates will increase to 20 million by the year 2020 (Sikora, 1999). In 1995, malignant neoplasm is the major cause of death (45%) in Government Hospitals of Malaysia, which is 2.8 times higher than that of heart disease (16%) (Malaysia’s Ministry of Health, 1995). Thus, despite advances in early detection and treatment, overall death rates from cancer have remained largely unchanged since the early 1970s, suggesting the need for a stronger research focus on prevention (Nestle, 1997).

Hepatocellular carcinoma is a cancer arising from the liver. Although the liver is made up of different cell types (e.g. Kupffer cells, hepatocytes, endothelial cells, sinusoidal and fat-storing cells) but the majority of primary liver cancers (over 90 to 95%) arises from hepatocytes and is called hepatocellular carcinoma (Fong, 2002). Hepatocarcinogenesis is a transformation process of normal liver cell into a cancerous cell which involved initiation, promotion and progression stages (Lai and Peter, 1999).

More than 3, 000 plant species have been used as anticancer agents (Lewis and Elvin-Lewis, 1977). This subject has received a lot of attention from both consumers and scientists during recent decades (Poppel and Berg, 1997). A lot of studies reported that various kinds of fruits, green and yellow vegetables, particularly cruciferous vegetables
have their anticancer activity towards human (Kusamran et al., 1998). In Malaysia, about 1200 plant species have been reported to have potential pharmaceutical value and some of these have been used as herbal medicine (Soepadmo, 1991). One example of medicinal plant found in Malaysia and Indonesia which has pharmacological potential is *Strobilanthes crispus*. In Indonesia, this plant has been used as antidiabetic, diuretic, antilytic and laxative agent (Sunarto, 1977).

In this study, the anticancer effect of *Strobilanthes crispus* was investigated, *in vivo*. The anticarcinogenic effect of 5% (w/v) *Strobilanthes crispus* during hepatocarcinogenesis has been reported by Elizabeth, (1999) and at this concentration, the extract was found to be very effective in treating hepatocarcinogenesis. Another study by Suherman et al. (2004) who tested 1%, 2.5%, 5%, 7.5% and 10% doses of *Strobilanthes crispus* extract on rats during hepatocarcinogenesis reported that among the doses, 5% (w/v) is optimum for supplementation.

Thus, in this present study, 5% (w/v) *Strobilanthes crispus* crude extract was chosen to treat DEN/AAF induced hepatocarcinogenesis. Atomic absorption spectroscopy (AAS) was not chosen to analyze the elements in this present study because in a study by Maznah et al. (2000), AAS has been used to analyze elements in *Strobilanthes crispus* crude extract. Thus in this study, EFTEM and VPSEM was chosen because this electron microscopes can analyze the antioxidant element at ultracellular level in liver tissue and no previous study has been carried out using these hi-technology equipments so far. While, gas chromatography mass spectroscopy (GCMS) also was not chosen because this