UNIVERSITI PUTRA MALAYSIA

THE NUTRITIONAL AND ANTINUTRITIONAL COMPOSITION OF STROBILANTHES CRISPUS (L.) BREMEK AND ITS ANTICANCER EFFECT DURING HEPATOCARCINOGENESIS

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FPSK (M) 1999 3

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By

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Medicine and Health Sciences Universiti Putra Malaysia

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UPM

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LIST OF ABBREVIATIONS

AAF	2-Acetylaminofluorene
AAS	Atomic absorption spectrophotometry
AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
BSA	Bovine serum albumin
CDNB	1-Chloro-2,4-dinitrobenzene
DEA	Diethanolamine
DEN	Diethylnitrosamine
DNA	Deoxyribonucleic acid
DTNB	Dithionitrobenzoic acid
EGCG	Epigallocatechingallate
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
FTC	Ferric thiocyanate
GGT	Gamma glutamyl transpeptidase
GL	Glycyrrhizin
GSH	Glutathione
GST	Glutathione S-transferases
H&E	Hematoxylin and eosin
HIV	Human immunodeficiency virus
HPLC	High pressure liquid chromatography
NNC	N-nitroso compounds
PNP	p-nitrophenol
PNPP	p-nitrophenyl phosphate
RDA	Recommended daily allowances
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
SCE	*Strobilanthes crispus* extract
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TEM	Transmission electron microscopy
TPP	Thiamin pyrophosphate
UDPGA	Uridyl diphosphoglucuronic acid
UDPGT	Uridyl diphosphoglucuronyl transferase

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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By

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Faculty: Medicine and Health Sciences

The nutritional and antinutritional composition of *Strobilanthes crispus* (L.) Bremek or *Saricocalyx crispus* (L.) Bremek (Acanthacea), and its anticancer effect during hepatocarcinogenesis were studied to investigate the possible cancer suppressive effect of the component existed in the leaves. The nutritional composition studied were of the proximate composition and antioxidant activity of ferric thiocyanate and thiobarbituric acid methods. The antinutritional factors studied were catechin, tannin, caffeine and alkaloid. All data were compared to Yerbamate, green tea, black tea and Indian tea. The leaves contained high amount of water soluble vitamins and total ash leading to high amount of minerals such as potassium, calcium, sodium, iron and phosphorus. The leaves contained low level of antinutritional factors contributing to better absorption of iron and water soluble vitamins. Its catechins showed highest antioxidant activity when compared to Yerbamate and Vitamin E. Consumption of the leaves extract daily (5 g/day) could contribute to the additional nutrient and antioxidant

needed in the body, especially iron. As of the *in vivo* study, hepatocarcinogenesis was monitored in rats according to Solt and Farber (1976). The 6 – 8 weeks old (150 – 200 g) Sprague Dawley rats were treated with 200mg/kg diethylnitrosamine (DEN) and 0.02% of 2-acetylaminofluorene (AAF) without partial hepatectomy. Five percent (w/v) of *Strobilanthes crispus* extract (SCE) was used compared with 5mg/100ml or 0.005% (w/v) Glycyrrhizin. The severity of neoplasia was studied by histological evaluations, body and liver weight profile and tumour markers. Histological evaluations showed loss of normal cell organization when carcinogens were introduced into the body. The neoplastic lesion was ameliorated significantly ($p<0.05$) with the treatment of SCE than Glycyrrhizin. The markers used were gamma glutamyl transpeptidase (GGT), uridine diphosphoglucuronyl transferase (UDPGT), glutathione S-transferases (GST), glutathione (GSH) and alkaline phosphatase (ALP). Rats treated with DEN/AAF had significantly ($p<0.05$) elevated results in all tumour markers except ALP, supported by body weight profile and the relative liver weight. SCE showed significant suppressive effect towards cancer cells, better than Glycyrrhizin at dosage level. Moreover, there was no evident suggesting side effects of SCE towards normal cells. Higher concentration (15 mg/100 ml or 0.015%) of Glycyrrhizin is believed to suppress cancer more effectively than 0.005% (w/v).

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KOMPOSISI NUTRIEN DAN ANTINUTRIENT *Strobilanthes crispus* (L.) BREMEK DAN KESAN ANTIKANSER SEMASA HEPATOCARCINOGENESIS

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Komposisi nutrien dan antinutrien daun *Strobilanthes crispus* (L.) Bremek atau *Saricocalyx crispus* (L.) Bremek (Acanthacea) dan kesan antikansernya semasa hepatokarsinogenesis telah dikaji untuk menentukan kemungkinan kesan penumpasan kanser oleh komponen di dalam ekstrak daun tersebut. Komposisi nutrien yang dikaji ialah komposisi proksimat dan aktiviti antioksidan iaitu kaedah ferik tiosianate dan asid tiobarbiturik. Faktor antinutrien yang dikaji ialah katekin, tannin, kafein dan alkaloid. Semua data dibandingkan dengan Yerbamate, teh hijau, teh perang dan teh India. Daun ini mengandungi kandungan vitamin larut air yang tinggi dan kandungan abu yang tinggi yang menyebabkan ketinggian kandungan mineral seperti kalium, kalsium, natrium, ferum dan fosforus. Komposisi antinutrien di dalam daun ini adalah rendah yang mengalakkan penyerapan ferum dan vitamin larut air. Katekinnya menunjukkan aktiviti antioksidan yang tinggi berbanding dengan Yerbamate dan Vitamin E. Pengambilan harian (5 g/hari) ekstrak daun ini boleh membantu menambahkan nutrien,

terutama zat besi, dan antioksidan yang diperlukan oleh tubuh. Kesan ke atas hepatokarsinogenesis *in vivo* pula dilakukan mengikut kaedah Solt dan Farber (1976). Kanser pada tikus *Sprague Dawley* berumur 6 – 8 minggu (150 – 200 g) diaruh dengan 200 mg/kg dietilnitrosamin (DEN) dan 0.02% 2-asetilaminofluorin (AAF) tanpa hepatektomi separa. Lima peratus (w/v) ekstrak *Strobilanthes crispus* (SCE) digunakan berbanding dengan 5 mg/100 ml atau 0.005% (w/v). Keterukan neoplastik dikaji dengan penilaian histologi, berat badan, berat relatif hati dan penanda tumor. Penilaian histologi menunjukkan kehilangan penyusunan sel-sel normal apabila karsinogen diaruh ke dalam badan. Lesion neoplastik didapati bertambah baik secara signifikan ($p<0.05$) dengan pemberian SCE daripada Glycyrrhizin. Penanda enzim yang dikaji ialah gamma glutamil transpeptidase (GGT), uridil difosfoglucuronil transferase (UDPGT), glutation S-transferase (GST), glutation (GSH) dan alkaline fosfat (ALP). Tikus yang diaruh dengan DEN/AAF mempunyai aras enzim penanda yang lebih tinggi secara signifikan ($p<0.05$) daripada normal kecuali pada ALP. Kenyataan ini disokong oleh perubahan berat badan dan berat relatif hati. SCE menunjukkan kesan penumpasan sel-sel kanser yang lebih baik daripada Glycyrrhizin pada kepekatan yang dikaji. Tambahan pula, tidak ada bukti yang menunjukkan kesan sampingan SCE terhadap sel-sel normal. Kepekatan Glycyrrhizin yang lebih tinggi (15 mg/100 ml atau 0.015%) dipercayai boleh menumpaskan kejadian kanser dengan lebih efektif berbanding dengan 0.005%.

CHAPTER I

INTRODUCTION

Cancer remains a hugely expensive public health problem in the world, both in economic terms and in terms of the amount of human suffering it produces. The past two decades have witnessed an explosion in the understanding of the molecular basis of malignant disease and in the rapid application of basic research concepts to the clinical arenas of diagnosis. Cancer has been the second leading cause of death in the United States for decades. Although heart disease leads cancer by more than 300 000 deaths per year, cardiovascular deaths are declining, a realization that had led some to project that cancer deaths will predominate by the turn of the century (Weiss, 1993). In Japan, cancer has become the greatest cause of mortality since 1981. It accounts for one-fourth of all deaths in the population (Fujiki *et al.*, 1992; Namiki, 1994). Malaysia's Ministry of Health (1995) has statistically reported that malignant neoplasm (45%) is the major cause of death in Government Hospitals, which is 2.5 times higher than the heart diseases (16%).

Research data currently available support the position that close to about 80 – 90% of the forms of most human cancers are caused by exposure of individuals to environmental factors (Jensen & Madsen, 1988), which involved the three general classes of carcinogenic agents, i.e. radiations, viruses and chemicals, and combination thereof (Miller & Miller, 1981).

These are mainly due to life-style factors, such as smoking and dietary habits. Cigarette smoking is a major risk factor in the development of cancer of the lung, uterine, cervix, bladder, pancreas and kidney (Daly, 1993). Diet, which provides both carcinogens and anticarcinogens, is likely to be another major risk factor for cancer development (Ames, 1983) by modulating the metabolism of chemical carcinogens and influencing the selective development and growth of initiated cells or latent tumours to yield gross tumours (Miller & Miller, 1981). Humans are exposed concomitantly or sequentially to several carcinogens at low doses (Hasegawa *et al.*, 1991). Other factors involved in human cancer development include hormones, drugs and chronic irritation (Wakabayashi *et al.*, 1992).

Cancer development is a complicated process consisting of many steps namely initiation, promotion and progression, with multiple gene alterations found in human cancers. Many ways to prevent development of cancer have been proposed such as avoiding exposure to environmental carcinogens as far as possible and also to isolate an effective chemopreventive compound (Wakabayashi *et al.*, 1992) by using nontoxic anticancer agents, so-called cancer chemoprevention (Fujiki *et al.*, 1992).

Chemoprevention of cancer is a mean of cancer control in which the occurrence of this disease is prevented by the administration of one or several chemical compounds. Some are naturally occurring constituents of food; others are purely synthetic. Chemopreventive agents used must have

trivial or no toxicity and desirably effective in a variety of tissue sites (Wattenberg, 1992). Their common property is the ability to induce the activities of electrophile–detoxification systems (Davidson *et al.*, 1990) therefore, blocking one or more steps in the process of the carcinogenesis (Ito *et al.*, 1994).

Although recent advances in cancer diagnosis as well as in cancer therapy are really remarkable, cancer mortality will not easily be reduced (Fujiki *et al.*, 1992). Henderson *et al.* (1992) suggested that there should be an ongoing evaluation due to the continuing cancer burden and the relatively slow impact of proven cancer prevention and treatment strategies in reducing cancer mortality. It is important to identify safe and effective agents, which prevent the endogenous formation or enhance detoxification of carcinogens and/or inhibit tumour promotion and progression (Reddy & Rao, 1994).

Over the centuries, no fewer than 3000 plant species have been used to treat cancer (Lewis & Elvin-Lewis, 1997). Many recent studies indicate that among most plants tea extracts has the most potent anticarcinogenic agents, thereby raising much interest not only in Japan but also in USA & China (Reddy & Rao, 1994). Body of evidence obtained from *in vitro* and animal studies concerning potentially protective effects of tea is compelling and encompasses several important mechanisms that suggest possible beneficial effects of tea and tea polyphenols at most stages of cancer

development (Dreosti *et al.* 1997). Tea becomes a product of great value and its popularity as a non-intoxicating drink was enhanced as the official beverage replacing wine. It has now grown to become a beverage which is consumed by more than half the population of the world for reasons not only of its pleasant taste and refreshing action, but also because it is considered to be an elixit for all ills (Wickremasinghe, 1993). Its beneficial effects are achieved with a dose of five to ten cups of tea per day. Each cup of green tea is equivalent to 40 mg of green tea extract (50%). Therefore the recommended daily use is between 0.2 – 0.4 g of green tea extract (Hara, 1994). Subsequent to medicinal properties of tea, many new plants are introduced and studied to increase the discovery of natural product cancer chemotherapeutic agents (Suh *et al.*, 1995).

In this study, the anticarcinogenic effect of *Strobilanthes crispus* crude extract (SCE) during rat hepatocarcinogenesis was investigated. Application of body weight profile, histological evaluation, and enzyme tumour markers results were used in this *in vivo* study. Concurrently, nutrient compositions of the plant related to cancer prevention were also evaluated. The significance and the outcome of this study could be associated with prognosis, which is very important in a medical treatment management.

Although *Strobilanthes crispus* has already been used as antideuritic traditionally for many years but its nutritional composition has never been determined. A recent research by Kusumoto *et al.* (1992) found that this

plant is a potent relief drug possibly used for AIDS and leukemia diseases, but further research on hepatocarcinogenesis or other diseases has not been done. Therefore, the objectives of this study were:

1) To evaluate the nutrient composition of *Strobilanthes crispus* inclusive of the proximate analysis, vitamin content, mineral content, antioxidant activity, as well as the content of various antinutritional factors (cathechin, tannin, caffeine and alkaloid)

2) To determine the anticancer activity of crude water extract of *Strobilanthes crispus* on hepatocarcinogenesis *in vivo* by the measurements of the body weight, liver weight, histological examinations and enzyme tumour markers activity such as glutathione S-transferases (GST), gamma-glutamyl transpeptidase (GGT), uridine diphosphoglucuronyl transferase (UDPGT) and alkaline phosphatase (ALP), as well as the glutathione (GSH) substrate

3) To compare the anticancer activity of SCE with the commercial drug, glycyrrhizin (GL), thereof evaluating the approximate picture and posibility of SCE in suppressing liver cancer.

CHAPTER II

LITERATURE REVIEW

Cancer

Cancer is a cellular phenomenon (Brock & Madigan, 1991) of rapid and uncontrolled formation of abnormal cells in the body (Lewis & Elvin-Lewis, 1977). Most cells in matured animals, although alive, do not divide extensively, apparently because of the presence of growth-inhibiting factors, which prevent them from initiating cell division under a variety of pathological conditions. Under some conditions, growth inhibition is overcome and the cells begin to divide uncontrollably. This extensive cellular growth is so excessive that the animal body is virtually consumed by cancer cells and the animal dies.

The development of cancer cells in many organs and tissues have altered growth requirements and continue to grow, piling up to form a small 'focus of growth' or 'foci' (Brock & Madigan, 1991). This associates with the prior appearance of new focal cell populations that are considered to be preneoplastic or premalignant precursor lesions (Farber *et al.*, 1976). Lesions can be divided into 3 groups; Group A as the non-neoplastic such as freckles and other familiar consequences of over-exposure of skin to sunlight, Group B as the benign neoplasms or papillomas which as non-invasive, and Group

C as the malignant tumours; constitute the sole manisfestation of disease (Lawley, 1987).

Avoidance or reduction of exposure to carcinogens and early diagnosis were the only hopes of reducing the incidence of cancer. Events that occur between exposure to carcinogens and the ultimate development or overt malignancy offer many opportunities for blocking or even reversing the neoplastic process (Talalay, 1992).

Carcinogenesis

Carcinogenesis is a study of the origin of cancer to determine the steps involved in conversion of normal cells to malignant cells (Marshall, 1993). It is a complex and protracted multistage process (Talalay, 1992) divided into at least three stages; initiation, promotion and progression (Ito *et al.*, 1983). Initiation is known as the subtreshold neoplastic state, promotion is where latent tumour cells were stimulated to proliferate to form visible tumours, and progression is known as the development of malignant tumours (Group C) from tissues bearing benign neoplasms (Group B) (Lawley, 1987).

One of the most promising and important ways to decrease human cancer mortality is through chemoprevention; though ideal chemopreventors

have not yet been developed. Chemoprevention is the process of inhibiting, delaying or reversing the process of carcinogenesis (Sharma *et al.*, 1994).

Hepatocarcinogenesis

The liver is often the first organ to be infected by metastasising cancer. Hepatocarcinogenesis or hepatocellular carcinoma or liver cancer is one of the most prevalent and deadly cancers worldwide (Kathryn *et al.*, 1997) which ranks seventeenth among cancers in order of frequency of occurrence. An increased risk for developing liver cancer is correlated with either virus infection or consumption of alcoholic beverages (Henderson *et al.*, 1992).

The liver has been studied intensively as a site for cancer development since 1935. It has been extensively utilized many times with many carcinogens for morphological, physiological and biochemical analysis of carcinogenesis because of (a) its size and its susceptibility to the induction of cancer, (b) growing body of knowledge about its cell biology, biochemistry and cell pathology, and (c) because of the ability to manipulate its state of proliferation (Farber & Cameron, 1980). Furthermore, the liver was convenient for quantitative biochemical analysis because it was considered to be a mass of relatively homogenous parenchymal cells and these cells has various marker enzymes (Kitagawa, 1976). The liver of the adult rat (150 – 200 g and larger) is ordinarily a quiescent organ with respect to cell