UNIVERSITI PUTRA MALAYSIA

NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND ANTICARCINOGENIC EFFECT OF TYPHONIUM FLAGELLIFORME (NICHOLSON 1029) EXTRACT IN RAT

THILAKAVATHY A/P KARUPPIAH

FPSK (M) 2000 3

NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND ANTICARCINOGENIC EFFECT OF *TYPHONIUM FLAGELLIFORME* (NICHOLSON 1029) EXTRACT IN RAT

By

THILAKAVATHY A/P KARUPPIAH

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

February 2000

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

NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND ANTICARCINOGENIC EFFECT OF *TYPHONIUM FLAGELLIFORME* (NICHOLSON 1029) EXTRACT IN RAT

By

THILAKAVATHY A/P KARUPPIAH

February 2000

Chairman: **Associate Professor Maznah Ismail, Ph.D.**

Faculty: **Medicine and Health Sciences**

Typhonium flagelliforme from the family Araceae is locally known as rodent tuber. It is being used traditionally in Malaysia to treat cancer. The major aim of this study was to investigate the effect of *Typhonium flagelliforme* crude extract on hepatocarcinogenesis in rats induced by diethylnitrosamine (DEN) and 2-acetylaminofluorene (AAF). Besides the *in vivo* study, the plant's nutrient and non-nutrient composition, and its antioxidant activity were also determined. *Typhonium flagelliforme* has carbohydrate (0.5% in leaves-stalks and 27.5% in tubers-roots) as its main constituent. It has high content of vitamin C (106.5 mg/100 g in leaves-stalks and 11.6 mg/100 g in tubers-roots) and potassium (1276.8 mg/100 g in leaves-stalks and 534 mg/100 g in tubers-roots). Alkaloid (0.4 mg/100 g in leaves-stalks and 0.9 mg/100 g in tubers-roots) and catechin (1.5% in leaves-stalks and 0.7% in tubers-roots) were found in this plant. The phytochemicals of *Typhonium flagelliforme* showed a greater antioxidant activity than vitamin E. The effect of *Typhonium flagelliforme* crude extract (0.1 ml/rat) on rat hepatocarcinogenesis was assessed by five different tumour

markers [gamma glutamyl transpeptidase (GGT), uridyl diphosphoglucuronyl transferase (UDPGT), glutathione S-transferase (GST), alkaline phosphatase (ALP) and glutathione (GSH)] and histological examinations. Glycyrrhizin [0.005% (w/v)] was used as a comparison to *Typhonium flagelliforme* crude extract. Administration of diethylnirosamine and 2-acetylaminofluorene (DEN/AAF) significantly increased the activities of plasma and liver microsomal GGT ($p<0.05$), liver microsomal UDPGT ($p<0.05$), liver GST ($p<0.05$), liver ALP ($p<0.001$) and the concentration of liver GSH ($p<0.01$) compared to control. Supplementation of *Typhonium flagelliforme* crude extract to normal rats did not give any effect towards the tumour markers. The crude extract administered to the DEN/AAF treated rats significantly decreased the activities of plasma and liver microsomal GGT ($p<0.05$), liver microsomal UDPGT ($p<0.05$), liver GST ($p<0.05$), liver ALP ($p<0.001$) and the concentration of GSH ($p<0.01$) when compared to the DEN/AAF treated rats. Glycyrrhizin, which was given to the DEN/AAF treated rats reduced all the tumour markers except ALP ($p>0.001$). Light microscopic examination showed that DEN/AAF caused hepatocytic dysplasia. Transmission electron micrographs showed organelles damage caused by DEN/AAF. No morphological and histological changes were seen on the cells and the organelles when the DEN/AAF treated rats were supplemented with the crude extract. These results suggested that *Typhonium flagelliforme* contained constituents that help in preventing liver cancer and its crude extract administered at this supplemented dose (0.1 ml) to the DEN/AAF treated rats reduced the severity of hepatocarcinogenesis better than glycyrrhizin [0.005% (w/v)].

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KOMPOSISI NUTRIEN, AKTIVITI ANTIOKSIDAN DAN KESAN ANTIKARSINOGENIK EKSTRAK *TYPHONIUM FLAGELLIFORME* (NICHOLSON 1029) PADA TIKUS

Oleh

THILAKAVATHY A/P KARUPPIAH

Februari 2000

Pengerusi: **Profesor Madya Maznah Ismail, Ph.D.**

Fakulti: **Perubatan dan Sains Kesihatan**

Typhonium flagelliforme dari famili Araceae dikenali sebagai keladi tikus di negara ini. Ia digunakan secara tradisional di Malaysia untuk merawat kanser. Objektif utama kajian ini adalah mengkaji kesan ekstrak kasar *Typhonium flagelliforme* terhadap hepatokarsinogenesis tikus yang diaruh dengan dietilnitrosamin (DEN) dan 2-asetilaminofluoren (AAF). Selain daripada kajian *in vivo*, komposisi nutrien dan antinutrien serta aktiviti antioksidan tumbuhan ini juga telah dikaji. Karbohidrat (0.5% dalam daun-batang dan 27.5% dalam ubi-akar) merupakan konstituen utama *Typhonium flagelliforme*. Ia mengandungi vitamin C (106.5 mg/100 g dalam daun-batang dan 11.6 mg/100 g dalam ubi-akar) dan kalium (1276.8 mg/100 g dalam daun-batang dan 534 mg/100 g dalam ubi-akar) yang tinggi. Alkaloid (0.4 mg/100 g dalam daun-batang dan 0.9 mg/100 g dalam ubi-akar) dan katekin (1.5% dalam daun-batang dan 0.7% dalam ubi-akar) terdapat dalam tumbuhan ini. Sebatian fitokimia *Typhonium flagelliforme* memberi kesan antioksidan yang lebih baik daripada vitamin E. Kesan ekstrak kasar

Typhonium flagelliforme pada hepatokarsinogenesis tikus diperiksa melalui lima penanda kanser [gama glutamil transpeptidase (GGT), uridil difosfoglukuronil transferase (UDPGT), glutation S-transferase (GST), alkalin fosfatase (ALP), glutation (GSH)] dan pemeriksaan histologi. Glycyrrhizin [0.005% (w/v)] digunakan sebagai pembanding kepada ekstrak kasar *Typhonium flagelliforme*. Pemberian dietilnitrosamin dan 2-asetilaminofluoren (DEN/AAF) meningkatkan dengan ketara aktiviti-aktiviti GGT plasma dan mikrosom hepar ($p<0.05$), UDPGT mikrosom hepar ($p<0.05$), GST hepar ($p<0.05$), ALP hepar ($p<0.001$) dan kepekatan GSH hepar ($p<0.01$) berbanding dengan kawalan. Suplementasi ekstrak kasar *Typhonium flagelliforme* kepada tikus normal tidak memberi sebarang kesan terhadap penanda-penanda kanser. Ekstrak kasar yang diberikan kepada tikus-tikus diaruh DEN/AAF mengurangkan dengan ketara aktiviti-aktiviti GGT plasma dan mikrosom ($p<0.05$), UDPGT mikrosom ($p<0.05$), GST hepar ($p<0.05$), ALP hepar ($p<0.001$) dan kepekatan GSH hepar ($p<0.01$) apabila dibandingkan dengan tikus-tikus diaruh DEN/AAF. Glycyrrhizin yang diberikan kepada tikus-tikus diaruh DEN/AAF mengurangkan kesemua penanda kecuali ALP ($p>0.001$). Pemeriksaan mikroskopi cahaya menunjukkan bahawa DEN/AAF menyebabkan displasia. Mikrograf transmisi elektron menunjukkan kerosakkan organel-organel disebabkan oleh DEN/AAF. Tiada perubahan morfologi dan histologi kelihatan pada sel-sel hepar dan organel-organelnya bagi tikus yang diaruh DEN/AAF dan disuplementasi dengan ekstrak kasar tumbuhan ini. Hasil kajian ini mencadangkan bahawa *Typhonium flagelliforme* mengandungi komponen-komponen yang dapat menghalang kanser hepar dan ekstrak kasarnya yang diberikan kepada tikus-tikus DEN/AAF pada dos ini (0.1 ml/tikus) mengurangkan keterukan hepatokarsinogenesis dengan lebih baik daripada glycyrrhizin [0.005% (w/v)].

ACKNOWLEDGEMENTS

Praise and Glory to God the Almighty, who has been my ever present Shield and Defender throughout the duration of my rigorous study in Universiti Putra Malaysia.

I offer my respectful obeisance unto my spiritual master, **His Divine Grace Yoga Jnana Sitthar Om Sri Rajayoga Guru**, who had made this possible **throughout**, without obstacles.

My deepest gratitude and appreciation to Associate Professor Dr. Maznah Ismail, Associate Professor Dr. Asmah Rahmat and Dr. Asmah Hj. Yahaya for their constructive criticisms, patience and effort, wise guidance and suggestions during the supervision of this thesis.

Sincere thanks go to my uncle and aunt, Mr. and Mrs. Muthuvelu, for all their help that they offered and still offering.

My thanks are also due to laboratory assistants especially Mrs. Siti Muskinah, Mr. Simon, Mrs. Maznah, Mrs. Aina, Mr. Ho and Miss Suleka for their assistance in analysis works.

I am also very grateful, to my dearest friends especially Miss Elizabeth Manickam, for their advice and help in various ways.

Last but not least, I would like to express my deepest gratitude to my beloved mother, brother and sister for their endless encouragement, patience, care and sacrifices that had helped me in my undertakings and to complete this research study successfully.

I certify that an Examination Committee met on 11th February 2000 to conduct the final examination of Thilakavathy a/p Karuppiah on her Master of Science thesis entitled "Nutritional Composition, Antioxidant Activity and Anticarcinogenic Effect of *Typhonium flagelliforme* (Nicholson 1029) Extract in Rat" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. The Committee Members for the candidate are as follows:

ABDUL SALAM ABDULLAH, Ph.D.
Professor,
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

MAZNAH ISMAIL, Ph.D.
Associate Professor/Head,
Department of Nutrition and Health Sciences
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ASMAH RAHMAT, Ph.D.
Associate Professor,
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ASMAH HJ. YAHAYA, Ph.D.
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor/Deputy Dean of Graduate School
Univerisiti Putra Malaysia

Date: 24 MAR 2000

This thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Master of Science.

KAMIS AWANG, Ph.D.
Associate Professor
Dean of Graduate School
Universiti Putra Malaysia

Date: 11 MAY 2000

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.

Thilathy

THILAKAVATHY KARUPPIAH

Date: 23/3/00

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AAF	2-acetylaminofluorene
ALP	Alkaline phosphatase
CDNB	1-chloro-2,4-dinitrobenzene
Ces	*Colocasia esculentum*
Ct	*Coleus tuberous*
DCNB	1,2-dichloro-4-nitrobenzene
DTNB	5'5'-dithio-bis-(2-nitrobenzoic acid)
DEN	Diethylnitrosamine
GGT	Gamma glutamyl transferase
GL	Glycyrrhizin
GSH	Glutathione
GST	Glutathione S-transferase
H&E	Hematoxylene and eosin
HCl	Hydrochloric acid
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HPO_3	Metaphosphoric acid
KCl	Potassium chloride
LH	Unsaturated fatty acid
$L^{\cdot}$	Lipid radical
LOH	Lipid peroxide

LOO	Peroxyl radical
MARDI	Malaysian Agriculture Research and Development Institute
NaOH	Sodium hydroxide
O_2	Oxygen
O_2^*	Superoxide
OH	Hydroxyl radical
TEM	Transmission electron microscopy
TFE	*Typhonium flagelliforme*
UDPGT	Uridyl diphosphoglucuronyl transferase
Xs	*Xanthosoma sagittifolium*

CHAPTER I

INTRODUCTION

Cancer is a disease, which today remains difficult to cure but is preventable by the administration of one or several chemical compounds. Ministry of Health Malaysia (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%).

Hepatocarcinogenesis or hepatocellular carcinoma or liver cancer is one of the most prevalent and deadly cancers worldwide (Kathryn *et al*., 1997). The liver is one of the most frequently damaged organs in the body, and it is indeed fortunate that it has an enormous functional reserve. Liver is an important metabolic organ. It is involved in the catabolism of many endogenous substances, and has the capacity to metabolise foreign carcinogens. Not only can reactions therein lead to direct-acting ultimate carcinogens but also additional reactions lead to conjugates which are in transport forms. Where specific tissues or cells have the enzymatic potential to split the active entities from their transport conjugates, cancer may result at such sites.

The sequence of events leading to tumour formation and the resulting cascading effects of the metastases has not been completely elucidated. What had been charted are various steps beginning with an insult to genetic cellular material (genotoxic event; known also as "initiation") and then to production of abnormal DNA non-genotoxic event: known as "promotion"). The transformation of the altered cell may lead to the proliferation of cells with invasive (malignant) or non-invasive (benign) qualities. From a confined region of tissue, cancer spreads to other tissues (developing secondary tumours or metastases), and finally, to an ultimate cascading effect of the tumourous cells (Nagy and Attaway, 1992).

Through animal studies, measuring tumour marker enzymes can identify the presence of cancer quantitatively by biochemical or immunochemical means in tissue or body fluids. This is further validated by histological examination for diagnosing neoplasia. Electron microscopy has made visible for the first time those cellular structures that are the morphological bases of metabolic process.

Human are exposed to large numbers of carcinogenic chemicals and other carcinogenic stimuli (i.e., ultraviolet light, radon, x-rays, etc.) in their daily life. Small amounts of many naturally occurring mutagens and carcinogens are ingested in our normal diet, and it is not known whether a lifetime of eating small amounts of these dietary carcinogens can cause or contribute to cancer in some people. In addition to the carcinogens and mutagens in our diet, we also ingest large numbers of naturally occurring anti-mutagens and anti-carcinogens. Epidemiological studies indicate that

dietary factors play an important role in the development of human cancer, and attempts to identify these naturally occurring dietary carcinogens and anti-carcinogens should lead to new strategies for cancer prevention.

If there is one aspect of medical practice that has united the physicians of the world throughout the millennia despite all their diverse opinions, is their reliance on our flora as a staple source of medicinal drugs. Virtually all early civilisations developed the use of plant drugs to a high degree. Traditional medicine is well known for its high nutritional value, as well as, its ability to cure various ailments. Treatments and remedies have varied greatly over the centuries; no fewer than 3000 plant species have been used by the laity to treat cancer (Lewis and Elvin-Lewis, 1977). Most of the early treatments had little or no effect on the disease, but perhaps they lessened suffering either physically or psychologically. Many herbal formulas were used in cancer treatment but none, as far as is known, was recorded as a cure.

In treating cancer, several plants are used in Malaysia traditionally, such as 'akar susun kelapa'(*Tabernaemontana divaricata)*, 'akar melur'(*Jasminum sambac*), 'bunga raya putih'(*Hibiscus rosa-sinesis)* and 'ubi bemban'(*Marantha arundinacea)*. One of the greatest advantages of herbal medicine as a therapy is that when administered correctly it is completely safe (Zakaria and Mohd., 1994).

Typhonium flagelliforme is a green plant about one foot tall. Its flower ends in a long filament resembling the tail of a mouse, hence its popular lay name rodent tuber. The plant grows readily in soft, damp shady areas. It has been used in traditional medicine for treating different types of malignancy.

Objectives

Even though *Typhonium flagelliforme* has been used traditionally to cure cancer and it is gaining popularity among the scientists, its nutritional and non-nutritional composition has never been reported. *In vivo* studies on cancer using this plant were not done yet. Therefore, the objectives of this study were:

1) To determine the nutrient and non-nutrient composition of *Typhonium flagelliforme* such as the proximate analysis, mineral, vitamins, alkaloid, tannin and catechin content.
2) To evaluate the antioxidant activity of different parts of *Typhonium flagelliforme.*
3) To determine the effect of the crude extract of *Typhonium flagelliforme* in suppressing the process of hepatocarcinogenesis in rats by assessing the body and liver weight, histological examinations (light and transmission electron microscopy), and tumour markers activities such as gamma glutamyl transpeptidase, glutathione S-transferase, alkaline phosphatase, uridyl diphosphoglucuronyl transferase and glutathione S-transferase dependent substrate, glutathione.
4) To compare the anticarcinogenic activity of *Typhonium flagelliforme* with glycyrrhizin, one of the drugs used to treat liver cancer.

CHAPTER II

LITERATURE REVIEW

Typhonium flagelliforme

Typhonium is derived from the word Typhon, a mythological giant: the name was given by the ancients to some Aroid. A genus embracing about thirteen species of stove, tuberous, parennial herbs, inhabiting tropical Asia, Australasia, and the Pacific Islands represents the family Araceae. The species known to cultivation are *T. Brownii*, *T. cuspidatum*, *T. divaricatum*, *T. diversifolium Huegelianum*, and *T. trilobatum.* They thrive in light, rich soil, and during the growing season require an abundant supply of water. After the leaves have died down, water must be withheld until growth recommences. The pots containing the tubers can be stored away in any dry, warm place (Nicholson, 1991).

Plants of *Typhonium flagelliforme* (Lodd.) Blume were previously described as *Typhonium divaricatum* (Nicholson and Sivadasan, 1981). In Malaysia and Singapore, *Typhonium flagelliforme* (Plate 1 and 2) is locally known as "rodent tuber" (Neoh, 1992). Characteristic of *Typhonium flagelliforme* are sterile flowers spreading, lower

ones spatulate but upper ones increasingly subulate; spathe pale greenish, extremely narrow and elongate and leaves usually narrowly hastate.

Rare fatty acids i.e. benzenetridecanoic acid and benzenetridecanoic acid methyl ester, from this species were isolated (Chen *et al.*, 1997). Preliminary tests showed that *Typhonium flagelliforme* suppress cancer cells such as leukaemia, colon carcinoma and melanoma. The extract, which is mixed with honey and drunk immediately, may be taken as single therapy or taken during the course of radiotherapy or chemotherapy. There are apparently no serious long-term side effects (Neoh, 1992).

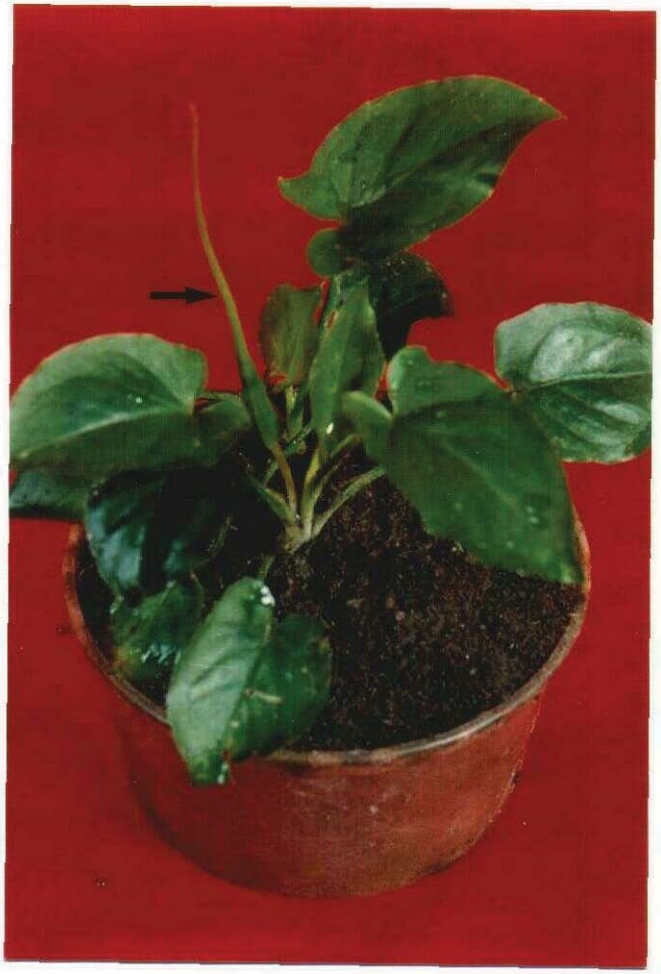

Plate 1: *Typhonium flagelliforme*. (→) Flower Resembling the Tail of a Mouse.