EFFECT OF HERBAL PLANTS ON OXYGEN RADICAL INDUCED LIPID PEROXIDATION IN LIVER HOMOGENATE

CHAU ANN LIE

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I hereby declare that this dissertation is my original research work except for all the references which I have mentioned their sources in detail.

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ABSTRAK

Terdapat banyak penyelidikan mengemukakan penglibatan ROS dalam Ferric nitrilotriacetate (Fe-NTA) yang mengakitbatkan toxic pada hati berkenaan carcinogenesis padanya. Fe-NTA adalah agen nephrototoxic dan sebagai promoter hepatik tumor yang mengakitbatkan peroksidasi lipid dan kemusnahan DNA. Peroksidasi lipid and kemusnahan DNA adalah penunjuk utama bagi Fe-NTA yang mengakibatkan toxiciti dan boleh dikawal oleh antioxidant. Objektif kajian dalam penyelidikan ini adalah untuk menilai kesan-kesan tumbuhan herbal yang dipilih iaitu, *Orthosiphon stamineus, Lonicera japonica, Strobilanthes crispus* pada radikal oksigen yang mengakibatkan peroksidasi lipid pada hati tikus supaya dapat kesan yang berkemungkinan terhadap peroksidasi lipid. Penilaian peroksidasi lipid adalah dinilai dengan kadar thiobarbituric acid reacting substance (TBARS) yang dihasilkan dan dibaca sebagai malondialdehyde (MDA). Keputusan yang dibaca sebagai n mol MDA hasil/h/g/tisu pada 535 nm dengan menggunakan spectrophotometer. Tindak balas antara hati dengan hydrogen peroksida dan Fe-NTA menyebabkan peroksidasi lipid pada hati sebanyak 2.3 ganda dan dibandingkan dengan kawalan. Keputusan menunjukkan bahawa pada dose yang tinggi dengan kandungan tumbuhan ekstrak ((50 ug/ml) yang digunakan, semakin tinggi kawalan terhadap peroksidasi lipid yang diperhatikan ($P < 0.05$). kawalan dengan tumbuhan ekstrak telah menunjukkan pengurangan peoksidasi lipid (43%-57%) seperti yang dibaca dengan MDA. Kawalan bagi peroksidasi lipid adalah bergantung kepada dose yang digunakan. Dapat disimpulkan bahawa, tumbuhan herbal tersebut berkemungkinan berguna sebagai modulator Fe-NTA dan hydrogen peroksida yang mengakibatkan peroksidasi lipid disebabkan oleh kehadiran komponen polyphenolic yang memberikan kesan-kesannya.

ABSTRACT

Several studies have shown the involvement of ROS in iron nitrilotriacetate (Fe-NTA)-induced liver toxicity, which may have relevance to its carcinogenicity. Fe-NTA is a potent nephrotoxic agent and induces acute and subacute renal proximal tubular necrosis and hepatic tumor promoter which are known to cause lipid peroxidation and DNA damage. Lipid peroxidation and DNA damage are the principal manifestation of Fe-NTA induced toxicity, which could be mitigated by antioxidants. Therefore, the aims of this study was to evaluate the effect of selected herbal plants viz. *Orthosiphon stamineus., Lonicera japonica., Strobilanthes crispus.*, on oxygen radical-induced lipid peroxidation in rat liver homogenate for a possible protection against lipid peroxidation. The determination of lipid peroxidation was done by measuring the rate of production of thiobarbituric acid reacting substance (TBARS) expressed as malondialdehyde (MDA) equivalents and the results were expressed as n mol MDA formed/h/g tissue at 535 nm using a spectrophotometer. Incubation of liver homogenate with hydrogen peroxide (40mM) in the presence of Fe-NTA (0.1 mM) induces hepatic lipid peroxidation to about 2.3-fold as compared to saline treated control. The results demonstrate that at the highest dose of plant extract (50 ug/ml) used, higher inhibition was observed ($P < 0.05$). In lipid peroxidation protection studies, these plant extract treatment showed a dose-dependant inhibition (43%-57%, $P < 0.05$) of Fe-NTA and hydrogen peroxide – induced lipid peroxidation as measured by MDA. The decrease in the enhancement of lipid peroxidation depends on the dose used. It is concluded that these herbal plants may be a useful modulator of Fe-NTA and hydrogen peroxide-induced lipid peroxidation due its presence of polyphenolic compound might be responsible for such an effect.

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%	percent
-	minus
±	plus minus
℃	degree Celsius
M	Mol
g	gram
mg	miligram
cm	centimeter
nm	nanometer
mM	miliMol
n mol	nanomol
ml	milliliter
µl	microliter
µg/ml	microgram permililiter

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CHAPTER 1

INTRODUCTION

1.1 Lipid Peroxidation

Lipid peroxidation has been shown to be a great importance in mammalian physiology and pathophysiology in past three decades. Increased lipid peroxidation is generally believed to be important underlying cause of initiation of oxidative stress related various tissue injury and cell death, and further progression of many acute and chronic diseases. The potentially reactive derivatives of oxygen, ascribed as reactive oxygen species (ROS) such as O^{-}_{2}, H_2O_2 and OH, are continuously generated inside the human body as a consequences of exposure to a plethora of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer. Under normal circumstances, the ROS generated are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present. Detrimental effects caused by

ROS occur as a consequence of an imbalance between the formation and inactivation of these species. However, owing to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is hampered favoring the ROS upsurge that culminates in oxidative stress. Oxidative stress is through a series of events, dysregulate cellular physiology and its sustained presence may lead to pathogenesis of several chronic ailments (Nakayama *et al*., 1983; Fridovich 1988; Floyds 1990). The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, mitochondria, lipoproteins and DNA (Farber, 1994). This oxidative damage is a crucial etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, increased membrane permeability, neurodegenerative diseases and also in the ageing process (Hogg, 1998 and Pong, 2003). Therefore, inhibition of oxidative lipid damage may be one of the strategies in chemoprevention of large number of clinical disorders.

Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is warranted. The human body has evolved a large array of endogenous antioxidant defenses against oxidative stress including antioxidant enzymes such as superoxide dismutase, catalase, various peroxidases and certain small molecules with antioxidant activity such as glutathione (Rana *et al*., 2002), the hormone melatonin (Reiter, 1998), uric acid (Yu *et al*., 1998) and ceruloplasmin (Maxwell *et al*., 1997). These endogenous defenses do not completely protect against the sum of oxidative stresses challenging the body, and there is net oxidative damage, thus necessitating the need for

exogenous natural antioxidants. Epidemiological studies have found that the intake of antioxidants such as vitamin C reduces the risk of coronary heart disease and cancer (Marchioli *et al.*, 2001). The antioxidant may mediate their effect by directly reacting with ROS, quenching them or chelating the catalytic metal ions (Robak and Marcinkiewiez, 1995). Several synthetic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commercially available but are quite unsafe and their toxicity is a problem of concern. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant and chemopreventive ability that may be used for human consumption.

1.2 Sabah Herb Plant

Sabah is known for its mega diversity and is the third biggest in the world, which is very rich with plant resources. Most of the plant resources are still unknown or undetermined. Chang & Rasadah (1997) reported that there are 12,000 species of Angiosperm plants in Malaysia, of which about 1,200 species are believed to contain medicinal properties. Meanwhile, Sabah has the most prominent flora and this is the natural feature of this state. According to YB Datuk K.Y.Mustafa from Sabah State Government, Sabah is found to have more than 10,000 species of wild plant species and about more than 4,000 types have already been identified (Daily Express 2004). Medicinal plants that are used in traditional healing by the various ethnic groups in Malaysia have not yet been scientifically proven and many of these herbal plants have received little or no attention

from natural product chemists. In addition, very few medicinal plants are sold in market due to the fact that the properties of many local plants are not known. The lack of laboratory facilities and shortage of well trained researchers are the main factors which have contributed to the scarcity of research in the chemistry and uses in pharmacy and medicine of medicinal plants in Sabah. Medicinal plants have been used widely to cure diseases among the local people since early ago and from time to time (Kulip, 1997).

Various parts of the plants are involved such as leaves, roots and bark of the plants. This is due to the availability of them throughout the year compared to the flowers, fruits or seeds which can be found in certain time in the year. Medicinal plants are used internally or externally. For internal uses, plants are used to treat illness such as asthma, ulcers, fever, inflammation, pain, diabetes, hypertension, kidney and bladder problems and are boiled and drank as tea. While for external uses, plants are crushed and mixed with oil or water into paste and applied on to affected areas. For external usages, plants are used to treat stomach ache, cut or wound etc. Therefore, keeping this in a view, the present study aims to evaluate the effect of selected herbal plants viz. *Orthosiphon stamineus., Lonicera japonica., Strobilanthes crispus.,* on oxygen radical-induced lipid peroxidation in rat liver homogenate. The selection of these plants is based on documents where they have reported medicinal importance of these plants (Asmah *et al.*, 2006; Khamsah *et al.*, 2006).

1.3 Objectives

The aims of this study are:

(I) To prepare crude extract of selected herbal plants viz. *Orthosiphon stamineus., Lonicera japonica., Strobilanthes crispus*

(II) To study oxygen radical mediated-lipid peroxidation in rat liver homogenate

(III) To evaluate the effect of crude extract of herbal plants viz. *Orthosiphon stamineus., Lonicera japonica., and Strobilanthes crispus* on oxygen radical-mediated lipid peroxidation in rat liver homogenate by measuring the rate of production of TBARS (expressed as malondialdehyde equivalents)

CHAPTER 2

LITERATURE REVIEW

2.1 Selected Herbal Plants

2.1.1 Black Face General (*Strobilanthes crispus*)

Strobilanthes crispus, the Black Face General or commonly known as Peach Beling by locals, derived from the member of Acanthaceae family. The name probably originates from the dark colour of the leaf infusion. It is a woody spreading shrub that can easily reach to more than a meter in height (Wilson Wong, 2006). The dark green leaf is elliptical in shape with attractive glossy sheen. They are also rough and tough. In addition, they occur in pairs that grow on direct opposite sides of them, which is a characteristic of plants belonging to Acanthaceae family (Wilson Wong, 2006). It rarely produces flowers and the flowers are yellow in appearance. The *Strobilanthes crispus* has been in the limelight in recent years for its anti-cancer properties (Wilson Wong, 2006). Locals have

been reported to use the herb for the treatment of uterine fibroids and leukemia. The plant has long been used for the treatment of snake bites, kidney stones and diabetes in Indonesia (Wilson Wong, 2006).

Strobilanthes crispus has been used traditionally as antidiabetic, diuretic, antilytic, and laxative and has been proven scientifically to possess high antioxidant activity, anti-AIDS, and anti-cancer (Asmah *et al.*, 2006). The water extract of *Strobilanthes crispus* inhibits the proliferation of retrovirus which indicates an agent in viral disease such as acquired immune deficiency syndrome (AIDS) and adult T-cell leukemia (Kusumoto *et al.*, 1992). *Strobilanthes crispus* is also very rich in minerals like potassium, calcium, sodium, iron and phosphorus. Besides that, this herb contained high amount of vitamin C, B_1, B_2 which contribute high antioxidant activity. Other composition such as catechins, caffeine and tannin are also available (Ismail *et al.*, 2000). Recent study reveals that *Strobilanthes crispus* tea contained high amount of mineral content, phenolic content and displayed high antioxidant activity especially unfermented tea from old or matured plant (Abu Bakar *et al.*, 2004).

Figure 2.1 Black Face General (*Strobilanthes crispus*)

Figure 2.2 Black Face General Shrub

2.1.2 Misai Kucing / Cat's Whisker (*Orthosiphon stamineus)*

Orthosiphon stamineus, or locally known as Misai Kucing is a herbaceous shrub, which grows to a height of 1.5 meters. It is a member of the Lamiaceae family, a popular medicinal herb in South-East Asian countries like Malaysia and Indonesia. It grows well in wet soil and generally propagated vegetatively by cutting of mature stems. The leaves are simple, green, and glamorous that arranged in opposite pairs. It produces a unique blue to light violet flowers that looks like cat's whiskers (Lee and Chan, 2004).

Misai Kucing has been used for many centuries in South East Asian countries like Malaysia and Indonesia. It is appreciated for treating ailments of the bladder and kidney. Misai Kucing began to interest researchers as early as the beginning of the 20th century when this plant was introduced to Europe where it became a popular herbal tea. In Malaysia, it is also appreciated for its elegant unique flower and it is commonly seen growing in many home gardens.

Traditionally, *Orthosiphon stamineus* is used for treating diabetes, kidney and urinary disorders, high blood pressure and bone or muscular pain. It is widely used for the treatment of antiallergic, anti hypertensive, anti-inflammatory, and diuretic properties. (InduBala, 2000). It is used as a remedy for arteriosclerosis, kidney stones and nephritis. It is also used for treating gout, eruptive fever, hepatitis, menstrual disorder, influenza and rheumatism. In Malaysia, the leaves are used as diuretic and for treating catarrh of the