UNIVERSITI PUTRA MALAYSIA

BIOCHEMICAL AND MUTAGENIC EFFECTS OF 'KHAT' *(CATHA EDULIS)* IN RATS

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By

ADEL SHARAF AL-ZUBAIRI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

TO MY FAMILY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: **Associate Professor Patimah Ismail, PhD**

Faculty: **Medicine and Health Sciences**

Khat leaves were originally used as a stimulant and a remedy against diseases and khat chewing became a widespread habit that has a deep-rooted sociocultural tradition in Africa and the Middle East. The present study was undertaken to evaluate the biochemical and toxicological effects of crude *Catha edulis* extract sub-acute (7 weeks) administration and to investigate the biochemical, toxicological and mutagenic effects of *Catha edulis* crude extract sub-chronic (13 weeks) administration in rats. Seventy four Sprague-Dawley male rats were used in this study. The sub-acute treatment group (38 rats) was further divided into 4 groups (control group and 500, 1000 and 2000 mg/kg body weight treatment groups), while the sub-chronic treatment study group (36 rats) was subdivided into three further groups (control group and 1000 and 2000 mg/kg body weight treatment

groups). For genotoxicity assessment we used chromosomal aberrations assay (CAs) and single cell gel electrophoresis assay (SCGE), the comet assay.

Body weight changes and food consumption were found to be not significantly different among all treatment groups when compared to the corresponding controls. We estimated the lipid peroxidation products, as a biomarker of oxidative stress and free radical activity, malondialdehyde, MDA (measured as plasma TBARS) and the results in the sub-acute (7 weeks) treatment group were found to be non-significantly different compared to the control group, while in the 13 weeks treatment groups, MDA levels in the 1000 and 2000 mg/kg body weight treatment groups were found to be significantly ($P < 0.05$) lower, by 28% and 30% respectively, compared to the control group.

Lipid profiles, uric acid, albumin, liver enzymes activities and total and prostatic acid phosphatase (ACP) results in the sub-acute treatment groups were found to be non-significantly affected compared to the control group. In contrast testosterone was found to be 2.8 and 2.4 folds significantly higher ($P< 0.01$) in the 1000 and 2000 mg/kg body weight treatment groups respectively, compared to the control group. These levels were also found to be increased in the 500 mg/kg body weight treatment by 54% compared to the control group although the increase was not significant.

Results of serum total cholesterol and HDL cholesterol concentrations after 13 weeks treatment with *Catha edulis* crude extract were found to be significantly higher by 18% and 15% respectively ($P< 0.05$), in the 1000 mg/kg body weight treatment group compared to the control group. For the genotoxicity assessment tests we observed conflicting results between the CAs and comet assay. The results of CAs assay in the 2000 mg/kg body weight treatment group were found to be significantly higher (7.38%) compared to the control group (2.2%) ($P< 0.05$), while in the 1000 mg/kg body weight treatment group 2.5% aberrated metaphases were observed. On the other hand results of DNA damage in the comet assay were observed to show no significant difference between treatment and control groups. However the predominant chromosomal aberrations scored in the CAs were chromatoid gaps followed by chromatoid breaks. We can conclude that *Catha edulis* leaves contribute antioxidant properties due to its polyphenolic constituents as well as testosterone up-regulation. Further investigations are recommended to elucidate the effects of fresh leaves of *Catha edulis* on chromosomes and other biomolecules using molecular techniques.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebangai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN BIOKIMIA DAN MUTAGENIK 'KHAT' *(CATHA EDULIS)* DALAM TIKUS

Oleh

ADEL SHARAF AL-ZUBAIRI

Jun 2007

Pengerusi: **Profesor Madya Patimah Ismail, PhD**

Fakulti: **Perubatan dan Sains Kesihatan**

Daun Khat (*Catha edulis*) asalnya digunakan sebagai stimulasi dan penawar terhadap penyakit dan kunyahan daun khat telah menjadi amalan yang meluas yang juga merupakan amalan tradisi di Afrika dan Timur Tengah. Pada kajian yang telah dijalankan ke atas tikus bagi menentu kesan biokimia dan toksik terhadap pengambilan ekstrak *Catha edulis* dalam masa 7 minggu untuk rawatan sub-akut dan kesan biokimia dan kesan ketoksikan dan mutagenik terhadap pengambilan ekstrak *Catha edulis* dalam masa 13 minggu untuk rawatan sub-kronik. Sebanyak 74 tikus jantan jenis Sprague-Dawley telah digunakan di dalam kajian ini. Bagi kumpulan rawatan sub-akut (38 ekor tikus), ianya dibahagikan kepada 4 kumpulan iaitu (kumpulan kawalan dan kumpulan rawatan bagi kepekatan 500, 1000 dan 2000mg/kg

jisim badan). Manakala bagi kumpulan kajian rawatan sub-kronik (36 ekor tikus) kemudian dibahagikan kepada 3 lagi kumpulan (kumpulan kawalan dan kumpulan rawatan bagi kepekatan 100 dan 2000mg/kg jisim badan). Bagi penilaian genotoksisiti teknik Asai Aberasi Kromosom (CAs) dan Gel Elektroforesis Sel Tunggal (Pengasaian Komet) telah digunakan.

Terdapat perbezaan yang tidak signifikan bagi perubahan jisim badan dan pengambilan makanan jika dibandingkan dengan kumpulan kawalan. Produk peroksidasi lipid telah diukur sebagai biomarker kepada stress oksidatif dan aktiviti radikal bebas, malanodialdehid, MDA (diukur sebagai TBRAS plasma) dan keputusan untuk kumpulan rawatan sub-akut (7 minggu) didapati terdapat perbezaan yang tidak signifikan jika dibandingkan dengan kumpulan kawalan. Manakala bagi kumpulan rawatan untuk 13 minggu pula, paras MDA untuk 1000 dan 2000 mg/kg jisim badan pula didapati signifikan ($P<0.05$) sebanyak 28 peratus dan 30 peratus berbanding dengan kumpulan kawalan.

Bagi keputusan profil lipid, asid urik, albumin, aktiviti enzim hati dan total dan prostatik asid fosfat (ACP) ke atas rawatan sub-akut didapati tidak signifikan jika dibandingkan dengan kumpulan kawalan. Walau bagaimanapun, testosterone didapati signifikan sebanyak 2.8 dan 2.4 lebih tinggi ($p<0.01$) bagi 1000 dan 2000 mg/kg jisim badan berbanding dengan

kumpulan kawalan. Paras ini juga didapati meningkat sebanyak 54 peratus bagi 500 mg/kg jisim badan jika dibandingkan dengan kumpulan kawalan walaupun peningkatan itu tidak signifikan.

Bagi keputusan total kolesterol dan HDL kolesterol, selepas 13 minggu rawatan menggunakan *Catha edulis* didapati signifikan sebanyak 18 peratus dan 15 peratus lebih tinggi, ($p<0.05$) untuk 1000 mg/kg jisim badan jika dibandingkan dengan kumpulan kawalan. Bagi ujian penilaian genotoksisiti terdapat perbezaan keputusan antara CAs dan pengasaian komet. Keputusan pengasaian CAs dalam 2000 mg/kg jisim badan kumpulan rawatan didapati mempunyai signifikan yang tinggi (7.38 peratus) jika dibandingkan dengan kumpulan kawalan (2.2 peratus) ($P<0.05$), manakala bagi kumpulan rawatan untuk 1000 mg/kg jisim badan didapati sebanyak 2.5 peratus aberasi metafasa. Sementara itu, keputusan untuk kerosakan DNA dalam pengasaian komet, mendapati tiada perbezaan yang signifikan di antara kumpulan rawatan dan kumpulan kawalan. Bagaimanapun, predominasi untuk aberasi kromosom di dalam CAs ialah ruang chromatoid diikuti dengan perpecahan chromatoid. Kesimpulannya, *Catha edulis* mungkin menyumbang sebagai bahan antioksidan berdasarkan kandungan polifenolik juga sebagai regulasi testosteron. Kajian yang lebih mendalam dicadangkan untuk memastikan kesan daun *Catha edulis* ke atas kromosom dan biomolekul yang lain menggunakan teknik molekul.

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ACP	Acid Phosphatase
ACTH	Adrenocorticotrophic Hormone
ALP	Alkaline Phosphatase
ALT	Alanine amintransferase
AMI	Acute Myocardial Infarction
c-AMP	cyclic-Adenosine Monophosphate
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
CAs	Chromosomal Aberrations
CAT	Catalase
CB	Cytochalasin B
CCD	Charge-coupled Device
CDKs	Cyclin-Dependent Kinases
CGH	Comparative Genomic Hybridization
CHO	Chenese Hamaster Ovary
CNS	Central Nervous System
COL	Cholchicine
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic Acid
DSB	Double Strand Breaks

EDTA	Ethylenediamine Tetracetate
FBS	Fetal Bovine Serum
GGT	Gamma Glutamyl Transpeptidase
GK	Glycerol Kinase
GPO	Glycerol Phosphate Oxidase
GPx	Glutathione Peroxidase
GST	Glutathione-S Transferase
H_2O_2	Hydrogenperoxide
HDL-chol	High Denisty Lipoprotein cholesterol
IFCC	International Fedration for Clinical Chemistry
LDH	Lactate Dehydrogenase
LDL-chol	Low Density Lipiprotein cholesterol
LPL	Lipoprotein Lipase
MDA	Malonydialdehyde
M-FISH	Multiplex Fluorescent Hybridization
MN	Micronucleus
NaOH	Sodium Hydroxide
OECD	Organization for European Cooperation and Development
PBS	Phosphate Buffered Saline
PCE	Polychromatic Erythrocyte
PEG	Polyethylene Glycol
PHA	Phytohaemagglutinin

PSA	Prostatic Specific Antigen
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SCE	Sister Chroatid Exchange
SCGE	Single Cell Gel Electrophoresis
SD	Standard Deviation
SKY	Spectral Karyotyping
SOD	Superoxide Desmutase
TBARS	Thiobarbituric Acid Reactive Substances
TEP	Tetraethoxy Propane
TG	Triglycerides
UA	Uric Acid
UDS	Unscheduled DNA Synthesis
UV	Ultraviolet
VLDL	Very Low Density Lipoprotein
WHO	World Health Oranization

CHAPTER 1

INTRODUCTION

Khat is the common name for the plant *Catha edulis* Forskal (Family: Celastraceae), a tree or large shrub growing in many countries of East and Central Africa and the Arabian Peninsula (Watt and Breger, 1962; Kalix, 1988). The young leaves and shoots (Khat) are chewed for their stimulating effects due to the plant's phenylalkylamines (Khatamines) particularly cathinone (Kalix and Braenden, 1985).

Khat grows wild in countries bordering the Red Sea and along the east coast of Africa. The people of these countries have chewed khat for centuries. There are several names for the plant, depending on its origin: chat, qat, qaad, jaad, miraa, mairungi, cat and catha. In most of the Western literature, it is referred to as khat. The leaves have an aromatic odour. The taste is astringent and slightly sweet. The plant is seedless and hardy, growing in a variety of climates and soils. Khat can be grown in drought areas where other crops have failed and also at high altitudes. Khat is harvested throughout the year. Planting is staggered to obtain a continuous supply

(Luqman & Danowski, 1976). Khat is mainly grown in Ethiopia, Kenya, Yemen, Somalia, Sudan, South Africa and Madagascar. It has also been found in Afghanistan and Turkestan.

Previously, khat leaves were available only near to where they were grown. Recently, improved roads and air transport have allowed a much wider distribution. Khat is harvested in the early hours of the morning and sold in markets in late morning. It is presented as a bundle of twigs, stems and leaves, wrapped in banana leaves to preserve freshness (Luqman & Danowski, 1976).

Khat is a drug of natural origin (Kalix *et al.*, 1991) that man has found for inducing pleasurable feelings and has become known and used worldwide, whereas the use of other drugs of natural origin having these properties remained more or less confined to the areas of their origin. The stimulating properties of the leaves of khat were probably known before those of coffee (El-Mahi, 1962). It was used in Yemen even before coffee (Lewin, 1931). Khat grows as an evergreen bush or tree, usually about 1-6 meters and even 25 meters high in favorable climates and soil conditions and frequently classified into several categories by the color of the branches and leaves