



UNIVERSITI PUTRA MALAYSIA

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

ADEL SHARAF AL-ZUBAIRI

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

By

ADEL SHARAF AL-ZUBAIRI

Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy

June 2007



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
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

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

Oleh

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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 (Pengasaan 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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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xx
 CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	9
Chemical Composition of Khat Leaves	9
Active Constituents of Khat	12
Pharmacokinetics and Mechanism of Action of Cathinone	16
Effects of Khat Administration	18
Effects of Khat Chewing on the CNS	19
Psychic Effects of Khat Chewing	21
Anorectic Effect of Khat Chewing	23
Analgesia Induced by Cathinone	24
Hyperthermia Induced by Khat Chewing	25
Effects of Khat on Cardiovascular System	26
Effects of Khat Chewing on Gastrointestinal Tract	28
Effect of Khat Chewing on Reproduction and Fetal Life	30
Toxic Effects of Khat	32
Metabolic Effects of Khat Chewing	36
Chromosomes	38
Gene Mutations	40
Structural Chromosome Aberrations	41
Mechanisms of chromosome aberrations	43
Numerical Chromosome Aberrations	45
International Regulations for Mutagenicity Testing	47
Aneuploidy	49
Mechanisms of Aneuploidy Induction	50



	Implications of Aneuploidy on Human Health	52
	Methods for Aneuploidy Testing	54
3	METHODOLOGY	64
	Experimental animals	64
	Plant material, khat leaves	65
	Study design	65
	Blood Sample collection	67
	Materials and Methods	68
	Comet assay	68
	Chromosome Aberrations Assay (CA)	74
	Biochemical Tests	78
	Statistical analysis	79
4	RESULTS	80
	The Sub-Acute Effects (7 weeks) Study Group	81
	Effects on Food Consumption and Weight Change	81
	Effects on Products of Lipid Peroxidation	83
	Effects on Lipid Profiles	85
	Effects on Uric Acid and Albumin	88
	Effects on Liver Enzymes	90
	Effects on Total Acid Phosphatase (ACP), Prostatic ACP and Testosterone	94
	The Sub-Chronic (13 weeks) Effects Study Group	98
	Effects on Food Consumption and Weight Change	98
	Effects on Products of Lipid Peroxidation	101
	Effects on Lipid Profiles	103
	Effects on Uric Acid and Albumin	106
	Effects on Liver Enzymes	108
	Effects on Total ACP, Prostatic ACP and Testosterone	112
	Genotoxic Effects of <i>Catha Edulis</i> Crude Extract Administration	115
	Chromosome Aberrations Assay	115
	Comet Assay (Single Cell Gel Electrophoresis Assay)	123
	Comparison between Sub-Acute and Sub-Chronic Effects of <i>Catha Edulis</i> Crude Extract Administration	125
5	DISCUSSION	131
	The Scope of This Thesis	131
	Khat Chewing Habit	132
	The Effects of Polyphenolics (Tannins)	133
	Effects of <i>Catha Edulis</i> (Khat) Crude Extract Administration On Lipid Peroxidation and Lipid Profiles in Rats	137



Effects of Khat Crude Extract Administration on Liver Enzyme	145
Reproductive Effects of <i>Catha Edulis</i> Administration	149
Genotoxic Effect of <i>Catha Edulis</i> (Khat) Crude Extract Sub-Chronic Administration in Rats	154
Measurement of DNA Damage with Comet Assay	154
Clastogenic Effects of Sub-Chronic <i>Catha Edulis</i> Extract Administration in Rats Using the Chromosomal Aberration Assay (CAs)	156
6 CONCLUSION	162
REFERENCES	166
APPENDICES	195
BIODATA OF THE AUTHOR	206
LIST OF PUBLICATIONS	207



LIST OF TABLES

Table		Page
1	Categories of comets of DNA damage	82
2	Plasma levels of MDA, Lipid profiles, Uric acid and Albumin after 7 weeks of <i>Catha edulis</i> crude extract administration. (Doses given in mg/kg body weight/day).	235
3	Serum liver enzyme activities and, Total and Prostatic ACP and Testosterone after 7 weeks of <i>Catha edulis</i> crude extract administration. (Doses given in mg/kg body weight/day).	236
4	Plasma levels of MDA, Lipid profiles, Uric acid and Albumin after 13 weeks of <i>Catha edulis</i> crude extract administration. (Doses given in mg/kg body weight/day).	237
5	Serum liver enzyme activities and, Total and Prostatic ACP and Testosterone after 13 weeks of <i>Catha edulis</i> crude extract administration. (Doses given in mg/kg body weight/day).	238
6	Percentages of chromosomal abnormalities and number of different types of abnormalities after 13 weeks crude khat extract sub-chronic administration in rat	151
7	Mean level of oxidative DNA damage in control, 1000 mg/kg bw and 2000 mg/kg bw treatment groups	157
8	Plasma levels of MDA, lipid profiles, uric acid, albumin, total ACP, prostatic ACP and testosterone in the 1000 mg/kg body weight 7 weeks treatment group compared to 13 weeks treatment group	163
9	Plasma levels of MDA, lipid profiles, uric acid and albumin in the 2000 mg/kg body weight after 7 weeks treatment with <i>Catha edulis</i> crude extract compared to 13 weeks treatment group.	164



LIST OF FIGURES

Figure		Page
1	The MDA standard curve	92
2	Weight changes in groups fed <i>Catha edulis</i> crude extract for 7 weeks	114
3	Plasma MDA levels after 7 weeks <i>Catha edulis</i> crude extract administration	116
4	Serum total cholesterol levels after 7 weeks of khat crude extract administration	118
5	Serum T.G. levels after 7 weeks of khat crude extract administration	118
6	Serum HDL cholesterol levels after 7 weeks of khat crude extract administration	119
7	Serum LDL cholesterol levels after 7 weeks of khat crude extract administration	119
8	Plasma levels of uric acid after 7 weeks of khat crude extract administration in the treated groups	121
9	Plasma levels of albumin after 7 weeks of khat crude extract administration in the treated groups	121
10	Plasma AST levels after 7 weeks crude khat extract administration.	123
11	Plasma ALT activity after 7 weeks <i>Catha edulis</i> crude extract administration.	123
12	Plasma LDH activity after 7 weeks <i>Catha edulis</i> crude extract administration	124
13	Plasma ALP levels after 7 weeks <i>Catha edulis</i> crude extract administration	124
14	Plasma GGT activity after 7 weeks <i>Catha edulis</i> crude extract administration	125



15	Total ACP in the group of rats fed with khat crude extract for 7 Weeks	128
16	Serum Prostatic ACP levels after 7 weeks crude khat extract administration	128
17	Serum testosterone levels after 7 weeks crude khat extract administration	129
18	Weekly weight gain changes in the group of rats fed <i>Catha edulis</i> Crude extract administration for 13 weeks	132
19	Plasma levels of MDA after daily oral <i>Catha edulis</i> crude extract administration for 13 weeks	134
20	Plasma levels of total cholesterol after daily <i>Catha edulis</i> crude extract administration for 13 weeks	136
21	Plasma levels of T.G. after daily <i>Catha edulis</i> crude extract administration for 13 weeks	137
22	Plasma levels of HDL cholesterol after daily <i>Catha edulis</i> crude extract administration for 13 weeks	138
23	Plasma levels of LDL cholesterol after daily <i>Catha edulis</i> crude extract administration for 13 weeks	139
24	Uric acid in the group of rats fed with khat crude extract for 13 Weeks	141
25	Serum albumin levels in rats fed with <i>Catha edulis</i> crude extract For 13 weeks	141
26	Serum levels of ALT activity after 13 weeks crude <i>Catha Edulis</i> administration	143
27	Serum AST levels after 13 weeks crude <i>Catha edulis</i> administration	143
28	Serum LDH after 13 weeks crude <i>Catha edulis</i> administration.	144
29	Serum ALP activity levels after 13 weeks crude <i>Catha Edulis</i> Administration.	144



30	Serum GGT activity levels after 13 weeks crude <i>Catha edulis</i> Extract administration	145
31	Total ACP in the group of rats fed with <i>Catha edulis</i> crude extract For 13 weeks	147
32	Prostatic ACP in the group of rats fed with <i>Catha edulis</i> Crude extract for 13 weeks	148
33	Plasma testosterone levels in the group of rats treated with <i>Catha Edulis</i> crude extract for 13 weeks	148
34	Percent chromosomal aberrations in treated groups compared To the control after 13 weeks of khat extract administration in Addition to types of aberrations	152
35	A well metaphase chromosomes spread of untreated Sprague-Dawley rat	153
36	Normal karyotype and a karyogram of Sprague-Dawley rat	154
37	Metaphase chromosomes spread with A, showing ascentric Fragment. B, showing a chromatid break as indicated by the circle	155
38	Metaphase spread with $2n = 42$ showing a chromatid Gap as in the Circled chromosome	156
39	Different types of comets examined	158
40	Differences in plasma levels of MDA after 7 weeks and 13 weeks <i>Catha edulis</i> crude extract administration. Plasma levels of MDA Were found to be lower in the treatment groups of 13 weeks Compared to those of 7 weeks treatment groups.	162



LIST OF ABBREVIATIONS

ACP	Acid Phosphatase
ACTH	Adrenocorticotrophic Hormone
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
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 ₂ O 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 Chromatid 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 Organization



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

