



*Ain Shams University
Faculty of Women for Arts, Science and Education
Biochemistry and Nutrition Department*

Impact of consuming the water extract of oleo-gum resin (*Boswellia carteri*) on colon cancer induced in rats

Thesis

Submitted for Faculty of Women, Ain Shams University
In Partial Fulfillment For the Requirements of Master Degree of
Biochemistry And Nutrition

By

Huda Mohamad Ismail Abu-Elfadl

B.Sc. in Biochemistry and Nutrition, Biochemistry and Nutrition
Department, Faculty of Women, Ain Shams University

Under supervision of

Prof.Dr. Mona Ahmed Sadek

Professor of Biochemistry and Nutrition, Biochemistry and Nutrition
department, Faculty of Women, Ain Shams University

Dr. Lamiaa Abdou El-Lateaf Barakat

Assistant Professor of Biochemistry and Nutrition, Biochemistry and
Nutrition department, Faculty of Women, Ain Shams University

2013

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



صدق الله العظيم
سوره طه (114)

A circular frame containing a bouquet of pink roses and baby's breath flowers. The roses are in various stages of bloom, and the baby's breath consists of small, delicate white flowers on thin green stems. The entire scene is set against a plain white background.

Dedication...

***This work is dedicated to my family;
my Father, my Mother, my sisters
"Shimaa", "Heba", & "Aya", and my
brother "Haitham".***

***Thanks for their love,
encouragement, continuous help and
support, I was always inspired to
complete this work.***



جامعة عين شمس
كلية البنات للآداب والعلوم والتربية
قسم الكيمياء الحيوية والتغذية

شكر و تقدير

أتوجه بالشكر للسادة الأساتذة الذين قاموا بالإشراف على
الرسالة وهم:-

1. **الاستاذة الدكتورة / منى أحمد صادق**
أستاذ الكيمياء الحيوية والتغذية بقسم الكيمياء الحيوية والتغذية
بكلية البنات- جامعة عين شمس.
2. **الدكتورة / لمياء عبد اللطيف بركات**
أستاذ مساعد الكيمياء الحيوية والتغذية بقسم الكيمياء الحيوية
والتغذية بكلية البنات- جامعة عين شمس.

Acknowledgement

First and foremost, thanks to **Allah** for most Merciful and most Gracious who gave me the ability to carry out this work.

I would like to express my sincere gratitude and thanks with love to **Prof.Dr. Mona Ahmed Sadek**, prof. of Nutrition, Biochemistry and Nutrition department, Women's college, Ain Shams University, not only for suggesting, planning the point of research and valuable supervision, but also for her great help, guidance, appropriate choice of the research topic, continuous encouragement, she tided me over many difficulties throughout this work. No words seen to be sufficient to describe, to her owe much.

I would like to express heartily thankful and thanks with love to **Dr. Lamiaa Abdou El Lateaf Barakat**, assistant Prof. of Biochemistry and Nutrition, Biochemistry and Nutrition department, Women's college, Ain Shams University, for helping me, sincere advice and supporting me during all steps of this work.

With great pleasure, I would like to express my sincere gratitude to the staff members of Biochemistry and Nutrition Department, College of women, Ain Shams University, for their encouragement to carry out this work. I also like to express my thanks with love to my friends for their help and support throughout this work.

Abstract

Some herbal plants play a nutraceutical role to improve health, ameliorate the oxidative stress and eliminate risks which brave quality of life of both healthy individuals and patients by decreasing the complications of their suffering diseases. Oleo-gum resins of *Boswellia carteri* are used as traditional medicine for reducing risks of a variety of lethal diseases as anti-inflammatory and anti-carcinogenic activities. In view of such activities of the oleo-gum resins and its components, the bioactive chemical compounds in the *Boswellia carteri* oleo-gum resins water extract were investigated and the effects of oral doses on colon cancer were tested through different biological and biochemical measurements.

A number of adult male albino rats were divided into 5 groups; 3 groups were given oral doses of *Boswellia carteri* water extract pre and/or post treatment of colon cancer induction with DNBC, in addition of 2 groups of healthy and malignant rats.

The chemical analysis of *Boswellia carteri* water extract revealed that each 100 ml of the prepared *Boswellia carteri* gum resins water extract contains 180 ± 3.0 mg as GAE for total phenols, 0.58 ± 0.01 mg as catechin equivalent for flavonoids, 7.6 ± 0.2 μ g of anthocyanins, 10 ± 0.7 mg as tannins, 1.05 ± 0.02 mg as saponins, 0.92 ± 0.02 mg as alkaloids. The tested water extract also showed high free radical scavenging activity; 1,1-diphenyl-2-picryl hydrazyl (DPPH) radicals was scavenged by about $94.1 \pm 1.8\%$. The biological trial showed that oral consumption of *Boswellia carteri* gum resins water extract before and after colon cancer induction (pre/post) caused significant enhancement in the nutritional parameters; animals food intake, feed efficiency ratio as well as the body weight ($P < 0.05$) as compared with malignant group. The water extract given as pre/post oral doses for malignant rats caused significant increment in the blood levels of hemoglobin, hematocrit, RBC's count, serum iron and lymphocyte percentage by 67.4%, 37.3%, 42.0%, 25.5% and 107.4% respectively whereas significant decrement in the WBC's count by 59.5% as compared with the control malignant group. Treatment of malignant rats with *Boswellia carteri* as pre/post doses caused suppression of oxidative stress status as significant reduction in the levels of serum malondialdehyde (MDA) and colonic tissue MDA and nitric oxide (NO), by about 64.5%, 66.7% and 49.9%, accompanied by increased levels of blood and tissue reduced glutathione (GSH) by 124.4% and 89.3%, with supporting the enzyme activities of erythrocyte superoxide dismutase (SOD) and serum catalase (CAT) by 25.8%, and 74.1% respectively when compared with the control malignant group ($P < 0.05$). Significant improvement was also found in the levels of blood proteins and blood lipids profile in malignant rats by oral doses of the water extract when compared with the control malignant group. Further, serum tumor markers as alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen-19.9 (CA-19.9), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and alkaline phosphatase (ALP) were reduced significantly by oral doses of the extract given before and after colon cancer induction by 68.1%, 26.1%, 48.8%, 37.4%, 61.1% and 13.9%, respectively as compared with malignant rats consumed the extract after colon cancer induction ($P < 0.05$).

List of contents

Subject	Page No.
Introduction.	1
Aim of the work.	4
Review of literature.	6
1. Cancer.	6
1.1. Colon anatomy.	11
1.2. Colorectal cancer.	12
1.3. Types of colon cancer.	24
1.4. Dietary intervention in prevention of colon cancer.	25
2. <i>Boswellia carteri</i> oleo-gum resins (luban dakar).	29
2.1. Safety of <i>Boswellia</i> gum resins extracts.	36
2.2. Anti-tumor effects of the bioactive constituents of <i>Boswellia carteri</i> oleo-gum resins water extract.	37
Materials and methods.	50
Materials.	50
1. <i>Boswellia carteri</i> oleo-gum resins.	50
2. Diet.	50
3. Animals.	52
4. Chemical used for cancer induction.	52
Methods.	53
1. Preparation of oleo-gum resins water extract.	53
2. Chemical constituents and antioxidant capacity of tested <i>Boswellia carteri</i> gum resins water extract.	53
2.1. Determination of total phenols content.	53
2.2. Determination of total flavonoids content.	54
2.3. Determination of the bioactive component tannins.	54
2.4. Determination of antioxidant activity.	54
2.5. Determination of the bioactive component saponins.	54

2.6. Determination of the bioactive component alkaloids.	54
3. Animal trial.	55
4. Biochemical measurements.	58
4.1. Whole blood measurements.	58
4.1.1. Hematological Measurements.	58
4.1.1.1. Hemoglobin concentration.	58
4.1.1.2. Hematocrit measurements.	59
4.1.1.3. Determination of serum iron concentration	60
4.1.1.4. Erythrocytes (RBCs) count.	61
4.1.1.5. Leukocytes (WBCs) count.	62
4.1.1.6. Erythrocyte Indices.	63
4.1.2. Determination of reduced glutathione content (GSH).	64
4.2. Red Blood Cells Measurement.	66
4.3. Serum measurements.	68
4.3.1. Determination of carcinoembryonic antigen (CEA), cancer antigen-19.9 (CA-19.9) and alpha-fetoprotein (AFP).	68
4.3.2. Determination of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) concentrations.	70
4.3.3. Determination of alkaline phosphatase (ALP) enzyme activity.	72
4.3.4. Determination of catalase (CAT) enzyme activity.	73
4.3.5. Determination of total proteins.	75
4.3.6. Determination of albumin.	76
4.3.7. Calculation of globulins.	77
4.3.8. Calculation of A/G ratio.	78
4.3.9. Determination of malondialdehyde (MDA).	78
4.3.10. Determination of total cholesterol (TC).	79

4.3.11. Determination of triacylglycerols (TAG).	80
4.3.12. Determination of high density lipoprotein-cholesterol (HDL-C).	82
4.3.13. Calculation of Low density lipoprotein-cholesterol (LDL-C).	83
4.3.14. Calculation of very low density lipoprotein-cholesterol (VLDL-C).	84
4.3.15. Calculation of the atherogenic index.	84
4.3.16. Calculation of the risk factor (LDL-C/HDL-C).	84
4.4. Colon tissue homogenate measurements.	85
4.4.1. Determination of nitric oxide.	
4.4.2. Determination of malondialdehyde (MDA).	86
4.4.3. Determination of reduced glutathione (GSH).	86
5. Statistical analysis.	87
Result and discussion.	
1. Active components of <i>Boswellia carteri</i> oleo-gum resins water extract.	87
2. Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on some biological parameters.	91
3. Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on hematological evaluation.	102
4. Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on some tumor markers.	113
5. Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on oxidative stress status in experimental animals.	123
6. Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum proteins.	136

7. Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on blood lipids profile.	144
Summary.	152
Conclusion and recommendations.	157
References.	158
Arabic summary.	

List of tables

Table No.	Subject	Page No.
(1)	Composition of the balanced diet.	50
(1-a)	Composition of minerals mixture (AIN-93).	51
(1-b)	Composition of vitamins mixture (AIN-93).	52
(2)	The active components of the tested <i>Boswellia Carteri</i> oleo-gum resins water extract.	90
(3-a)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on some nutritional parameters.	99
(3-b)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on relative organs weight.	100
(4-a)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on hematological evaluation.	111
(4-b)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on hematological evaluation.	112
(5)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on selected tumor markers.	120
(6-a)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on oxidative stress status in experimental animals.	132
(6-b)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on oxidative stress status in experimental animals.	133
(7)	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum proteins.	142

(8)	Effect of consuming <i>Boswellia carteri</i> oleo- gum resins water extract pre and/or post oral doses on blood lipids profile.	150
-----	---	-----

List of figures

Figure No.	Subject	Page No.
1	Flow chart depicting a simplified scheme of the molecular basis of cancer.	10
2	Large intestine.	11
3	Colorectal cancer.	12
4	Cancer cachexia.	15
5	Pathway to GI cancer through bile acid induced DNA damage.	17
6	The main steps that link inflammation to cancer. via free radical-mediated processes.	20
7	<i>Boswellia carteri</i> (luban dakar).	29
8	Chemical structures of boswellic acids.	31
9	Boswellic acids induce apoptosis via caspases activation.	40
10	The cell cycle.	42
11	The 2 colon sections of healthy rats (A) and malignant one (B).	55
12	Schematic diagram of the work groups design.	56
13	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on change in rats body weight.	101
14	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on relative weights of liver, spleen and colon.	101
15	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum carcinoembryonic antigen (CEA).	121
16	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum cancer antigen-19.9 (CA-19.9).	121

17	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum (A) Alpha-fetoprotein (AFP), (B) Alkaline phosphatase (ALP).	122
18	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum Interleukin-6 (IL-6) and Tumor necrosis factor- α (TNF- α).	122
19	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on (A) RBCs superoxide dismutase (SOD), (B) serum catalase (CAT) enzyme activities.	134
20	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on (A) blood glutathione (GSH), (B) colonic tissue glutathione (GSH).	134
21	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on (A) Serum malondialdehyde (MDA), (B) colonic tissue MDA.	135
22	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on colonic tissue nitric oxide (NO).	135
23	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum total proteins, albumin and globulin.	143
24	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on albumin/globulin ratio (A/G ratio)	143
25	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum TC and TAG concentrations.	151
26	Effect of consuming <i>Boswellia carteri</i> oleo-gum resins water extract pre and/or post oral doses on serum HDL-C, LDL-C and VLDL-C concentrations.	151

List of abbreviations

A/G ratio	Albumin/globulin ratio
AA	Acetic acid
AAP	4-aminophenazone
ACF	Aberrant crypt foci
ADP	Adenosine diphosphate
AFP	Alpha-fetoprotein
AI	Atherogenic index
AIN-93	American institute of nutrition
AK β BA	Acetyl-keto- β -boswellic acid
AKBA	Acetyl-keto boswellic acid
ALB	Albumin
ALP	Alkaline phosphatase
AOM	Azoxymethane
AOPP	Advanced oxidation protein products
AP-1	Activator protein 1
APaF-1	Apoptosis activating factor-1
APC	Adenomatous polyposis coli tumor suppressor gene
ATP	Adenosine triphosphate
BAE	Boswellia alcoholic extract
BAs	Boswellic acids
Bax	BcL-2-associated x protein
BCG	Bromocresol green
BcL2	B-cell lymphoma 2
BcL-x(L)	B-cell lymphoma-extra large
bFGF	Basic fibroblast growth factor
BWE	<i>Boswellia carteri</i> water extract
Bwt	Body weight
CA-19.9	Cancer antigen-19.9
Caspase	Cysteine-aspartic proteases
CAT	Catalase
CDK	Cyclin-dependent kinase
CE	Catechin equivalent
CEA	Carcinoembryonic antigen