



Ain Shams University
Women's College
Biochemistry and Nutrition
Departement

***“Effects of probiotics as one of functional foods to
reduce high risk of cardiovascular diseases and
colon cancer”***

Thesis

Submitted to Women's College, Ain Shams University
For Ph.D. Degree in Science
Biochemistry and Nutrition

By

Rania Desoki Mohamed Ibrahim

M. Sc. Degree in Science
Biochemistry and Nutrition

Under Supervision of

Prof. Dr. Mona Ahmed Sadek

Professor of Nutrition
Biochemistry and Nutrition Department
Women's college
Ain Shams University

Dr. Jehan Abd El-Razek Hasanen

Assistant Prof. of organic chemistry in department of chemistry
Science College, Suez Canal University

Dedication...

*This work is dedicated to my family;
My mother, my husband, my brother, my sister,
my daughter: Nadine & my son: Mohammed.*

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

To my father (covered with Allah mercy).

بسم الله الرحمن الرحيم

(و قالوا الحمد لله الذي
هدانا لهذا و ما كنا لنهتدي
لولا أن هدانا الله)

صدق الله العظيم ..؛

سورة الأعراف – الآية (43)

Acknowledgement

My very deepest gratitude and sincere appreciation goes to ***Prof. Dr. Mona Ahmed Sadek***, Professor 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, her valuable criticisms has been most helpful at all stages and continuous encouragement, she tided me over many difficulties throughout the work. No words seem to be sufficient to describe, to her owe much.

I am thankful to ***Dr. Jehan Abd El-Razek Hasanen***, Assistant Prof. of Organic Chemistry Department of Chemistry, Science College, Suez Canal University, for sincere advice and help during the practical part of this work.

With great pleasure, I would like to express my sincere gratitude to the staff members of Biochemistry and Nutrition Department, Women's College, Ain Shams University for their encouragement to carry this work.

Abstract

Some functional foods such as probiotics play a beneficial role functions in the treatment of some health problems and emollurate the oxidative stress mechanisms and other risks which improving the quality of life. Cardiovascular diseases as well as colon cancer are two of the most important health problems, so we interest to choose the two tested probiotics yoghurt and fermented kidney beans as biofunctional foods to reduce the high risk of these two diseases.

Chemical analyses of the two tested probiotics were determined. For biological and biochemical uses evaluations female Swiss albino mice (n = 160) were classified into two major sections. The first section subdivided into healthy and atherogenic mice groups (atherogenicity induced by HFHC diet); while the second section subdivided into healthy and carcinogenic mice (cancer induced by injection colon by 2×10^6 Ehrlich ascites tumor cells).

Results of the first section were as follows: Biochemical values of lipids profile revealed that, induction of atherogenicity caused a significant increase in TC, TG and bad cholesterol LDL-C by 55.07 %, 19 % and 305.50 % respectively comparing with healthy group, with significant decrease in good cholesterol HDL-C (25.80 ± 4.30 mg/dl, v.s 35.20 ± 6.10 mg/dl). Feeding on one of the two tested probiotics led to significant decrease in plasma lipids profile of treated atherogenic mice of TC, TG and bad cholesterol LDL-C except that of good cholesterol HDL-C. Values of liver TC and TG go hand in hand with the levels of plasma TC and TG. Moreover levels of liver GSH revealed the antioxidant status of the atherogenic case which was decreased significantly, while there was a significant modulation in case of consuming yoghurt and FKB. With respect to faecal bile acids excretion, results showed that, when atherogenic mice fed on supplemented diet with either yoghurt or FKB excreted more bile acids than those of untreated group, the percentage of increment were being 45.65 % and 53.21 % respectively. Microscopic examination of aorta of carcinogenic mice feeding on either yoghurt or fermented kidney beans showed decreases of ulcers of superficial epithelial cells and the extravasated cells caused by atherogenicity.

Results of the second section were as follows: Hemoglobin (Hb) concentration and percentage of hematocrit (Hct) in blood of carcinogenic mice were significantly increased by consuming one of the two tested probiotics. The increment were 44 % & 47 % respectively in case of Hb concentration and by 41.60 % & 46.80 % respectively in case of Hct compared to untreated carcinogenic mice. Results of numerical counts of RBCs and WBCs go hand in hand with values of Hb and Hct. With respect to oxidative stress status of healthy and carcinogenic mice, results showed that induction of cancer resulted in marked reduction in serum vitamin E, vitamin A and selenium and increment in serum MDA. The functional role of the two tested probiotics decreased the risk of oxidative stress in treated carcinogenic mice by significant increment in serum vitamin E, vitamin A and selenium and decrement of serum concentration of MDA which were 70.46 % and 71.38 % respectively. Results of numerical counts of dead tumor cells indicated that feeding on one of the two tested probiotics led to significant increases of % of dead tumor cells (81.09 ± 2.72 % & 78.89 ± 2.45 % respectively). Microscopic examination of sections of colon indicated that, feeding on one of the two tested probiotics lowers the number and size of the tumor cells, with signs of degeneration and necrosis. Although fermented kidney beans is more effective in which cells of much smaller size than in case of feeding yoghurt and the majority of neoplastic cells were degenerated and lost their nuclei.



جامعة عين شمس

كلية النبات للآداب و العلوم والتربية

قسم الكيمياء الحيوية و التغذية

"تأثيرات البروبيوتك كأحد الأغذية الوظيفية لخفض الخطورة العالية من الإصابة بأمراض القلب والأوعية الدموية وسرطان القولون"

رسالة مقدمة إلى كلية النبات – جامعة عين شمس
للحصول على درجة الدكتوراة في فلسفة العلوم في الكيمياء الحيوية والتغذية
مقدمة من

رانيه دسوقي محمد إبراهيم
ماجستير في العلوم في الكيمياء الحيوية والتغذية

تحت إشراف
الأستاذة الدكتورة / منى أحمد صادق
أستاذ التغذية بقسم الكيمياء الحيوية و التغذية
كلية النبات – جامعة عين شمس

الدكتورة / جيهان عبدالرازق حسنين
أستاذ مساعد الكيمياء العضوية بقسم الكيمياء
كلية العلوم – جامعة قناة السويس

2010

LIST OF CONTENTS

<i>Subjects</i>	<i>Page No.</i>
Introduction.....	1
Aim of the work.....	3
Review of Literature.....	5
Functional foods.....	5
Probiotics	13
Role of probiotics in health and diseases	24
Cholesterol-lowering effect of probiotics.....	24
Anti-Carcinogenic effect of probiotics.....	29
Yoghurt.....	40
Yoghurt as probiotic	49
Role of probiotic yoghurt in health and disease	49
Cholesterol-lowering effect of probiotic yoghurt.....	49
Anti carcinogenic effect of probiotic yoghurt	54
General safety of effects of probiotics	60
Materials and Methods.	70
Materials	70
Animals.....	70
Diets.....	70
Tested probiotics.....	71
Tumor cell line.....	71
Methods	72
<i>1- Preparations and evaluations of the two tested probiotics.....</i>	<i>72</i>
1.1. Preparation of yoghurt.....	72
1.2. Preparation of fermented kidney beans	74
1.3 Chemical evaluation of the two tested probiotics	76
• Determination of ash.....	76
• Determination of fats	76
• Determination of proteins	76

LIST OF CONTENTS (Cont...)

<i>Subjects</i>	<i>Page No.</i>
<ul style="list-style-type: none"> • Determination of moisture 77 • Determination of carbohydrates..... 77 • Determinations of vitamin E and vitamin A 77 • Determinations of zinc and selenium..... 77 	
2- Animal feeding evaluation.....	78
3- Biochemical measurements	84
<i>I-First section</i>	<i>84</i>
1. Plasma measurements	84
<ul style="list-style-type: none"> • Determination of total lipids..... 84 • Determination of total cholesterol (TC) 85 • Determination of triacylglycerols (TG)..... 87 • Determination of high-density Lipoprotein cholesterol (HDL-C)..... 90 • Determinations of very low and low density lipoproteins (VLDL-C) and (LDL-C)..... 92 • Determination of Atherogenic Index (A.I) 92 	
2. Liver measurements	93
<ul style="list-style-type: none"> • Determinations of total cholesterol and triacylglycerols.93 • Determination of reduced glutathione. 93 	
3. Determination of fecal bile acids	95
<i>II- Second section</i>	<i>96</i>
1. Measurements of hemoglobin and hematocrit	96
<ul style="list-style-type: none"> • Hemoglobin measurement 96 • Hematocrit measurement..... 97 	

LIST OF CONTENTS (Cont...)

<i>Subjects</i>	<i>Page No.</i>
2. Blood cells count	98
• Red blood cells count (RBCs).....	98
• White blood cells count (WBCs)	99
3. Evaluation of antioxidants status	100
• Determinations of serum vitamin E and serum vitamin A.	100
• Determinations of serum selenium and zinc.....	101
• Determination of serum malondialdehyde (MDA)...	101
4. Ascites tumor dead cells count.....	102
4- Statistical analysis	103
5- Microscopic examinations	103

Results and discussion

The chemical properties of tested probiotics.....	104
1. Yoghurt.....	104
2. Fermented kidney beans.....	105
1. Effects of consuming yoghurt or fermented kidney beans on plasma lipids profile and atherogenic index of atherogenic and healthy mice.....	108
2. Effects of consuming yoghurt or fermented kidney beans on liver cholesterol, triacylglycerols and reduced glutathione (GSH) of atherogenic and healthy mice.....	116
3. Effects of consuming yoghurt or fermented kidney beans on faecal bile acids of atherogenic and healthy mice.....	121
4. Effects of consuming yoghurt or fermented kidney beans on microscopic examination of aorta of atherogenic and healthy mice.....	125

LIST OF CONTENTS (Cont...)

<i>Subjects</i>	<i>Page No.</i>
5. Effects of consuming yoghurt or fermented kidney beans on hemoglobin, percentage of hematocrit and blood cell counts of carcinogenic and healthy mice.....	128
6. Effects of consuming yoghurt or fermented kidney beans on serum vitamin E, vitamin A, selenium and malondialdehyde (MDA) of carcinogenic and healthy mice.....	134
7. Effects of consuming yoghurt or fermented kidney beans on the viability of Ehrlich ascites of carcinogenic and healthy mice.....	139
8. Effects of consuming yoghurt or fermented kidney beans on microscopic examination of colon of carcinogenic and healthy mice.....	143

LIST OF TABLES

Table No.	Subjects	Page No.
(1)	Composition of the two diets used (g/100g diet).....	70
(2)	The experimental design of mice of the first section.....	82
(3)	The experimental design of mice of the second section.....	83
(4)	Chemical analysis of the tested yoghurt.....	106
(5)	Chemical analysis of the tested fermented kidney beans.....	106
(6)	Biochemical effects of consuming yoghurt or fermented kidney beans on plasma lipids profile and atherogenic index (A.I) of atherogenic and healthy mice.....	112
(7)	Biochemical effects of consuming yoghurt or fermented kidney beans on liver TC and TG of atherogenic and healthy mice.....	118
(8)	Biochemical effects of consuming yoghurt or fermented kidney beans on faecal bile acids of atherogenic and healthy mice.....	123
(9)	Biochemical effects of consuming yoghurt or fermented kidney beans on hemoglobin, hematocrit, RBCs and WBCs of carcinogenic and healthy mice.....	131
(10)	Biochemical effects of consuming yoghurt or fermented kidney beans on serum vitamin E, vitamin A, selenium and MDA of carcinogenic and healthy mice.....	136
(11)	Effects of consuming yoghurt or fermented kidney beans on the viability of Ehrlich ascites in carcinogenic and healthy mice.....	141

LIST OF FIGURES

Figures No.	Subjects	Page No.
(1)	Chemical composition of yoghurt.....	107
(2)	Chemical composition of fermented kidney beans.....	107
(3a)	Biochemical effects of consuming yoghurt or fermented kidney beans on plasma total cholesterol (mg/dl) of atherogenic and healthy mice.....	113
(3b)	Biochemical effects of consuming yoghurt or fermented kidney beans on plasma triacylglycerols (mg/dl) of atherogenic and healthy mice.....	113
(3c)	Biochemical effects of consuming yoghurt or fermented kidney beans on plasma VLDL-C (mg/dl) of atherogenic and healthy mice.....	114
(3d)	Biochemical effects of consuming yoghurt or fermented kidney beans on plasma LDL-C (mg/dl) of atherogenic and healthy mice.....	114
(3e)	Biochemical effects of consuming yoghurt or fermented kidney beans on plasma HDL-C (mg/dl) of atherogenic and healthy mice.....	115
(3f)	Biochemical effects of consuming yoghurt or fermented kidney beans on plasma AI of atherogenic and healthy mice.....	115
(4a)	Biochemical effects of consuming yoghurt or fermented kidney beans on liver total cholesterol (mg/g tissue) of atherogenic and healthy mice.....	119
(4b)	Biochemical effects of consuming yoghurt or fermented kidney beans on liver triacylglycerols (mg/g tissue) of atherogenic and healthy mice.....	119

LIST OF FIGURES (Cont....)

Figures No.	Subjects	Page No.
(4c)	Biochemical effects of consuming yoghurt or fermented kidney beans on liver GSH (mg/g tissue) of atherogenic and healthy mice.....	120
(5)	Biochemical effects of consuming yoghurt or fermented kidney beans on faecal bile acids (mg/100g-feces) of atherogenic and healthy mice.....	124
(6)	Aorta section of healthy mice.....	126
(7)	Aorta sections of untreated atherogenic mice.....	126
(8)	Aorta sections of atherogenic mice feeding on yoghurt.....	127
(9)	Aorta sections of atherogenic mice feeding on FKB.....	127
(10a)	Biochemical effects of consuming yoghurt or fermented kidney beans on hemoglobin concentrations (g/dl) of carcinogenic and healthy mice.....	132
(10b)	Biochemical effects of consuming yoghurt or fermented kidney beans on hematocrit (%) of carcinogenic and healthy mice.....	132
(10c)	Biochemical effects of consuming yoghurt or fermented kidney beans on red blood cells count (10^6 cu.mm) of carcinogenic and healthy mice.....	133
(10d)	Biochemical effects of consuming yoghurt or fermented kidney beans on white blood cells count (10^3 cu.mm) of carcinogenic and healthy mice.....	133
(11)	Biochemical effects of consuming yoghurt or fermented kidney beans on serum vitamin E (μ g/ml) _n of carcinogenic and healthy mice.....	137

LIST OF FIGURES(Cont....)

Figure No.	Subjects	Page No.
(12)	Biochemical effects of consuming yoghurt or fermented kidney beans on serum vitamin A ($\mu\text{g/ml}$) of carcinogenic and healthy mice.....	137
(13)	Biochemical effects of consuming yoghurt or fermented kidney beans on serum selenium ($\mu\text{g/ml}$) of carcinogenic and healthy mice.....	138
(14)	Biochemical effects of consuming yoghurt or fermented kidney beans on serum MDA of carcinogenic and healthy mice.....	138
(15)	Effects of consuming yoghurt or fermented kidney beans on the viability of Ehrlich ascites (%) of carcinogenic and healthy mice.....	142
(16)	Section of colon in healthy mice.....	145
(17)	Sections of colon in untreated carcinogenic mice.....	145
(18, 19)	Sections of tumor colon in untreated carcinogenic mice...	146
(20)	Sections of tumor colon in carcinogenic mice feeding on yoghurt.....	147
(21)	Sections of tumor colon in carcinogenic mice feeding on FKB.....	147

List of Abbreviations

ACF	Aberrant crypt foci.
AI	Atherogenic index.
AIW	American Institute of Nutrition.
APC	Atrial premature contraction.
CRC	Colorectal cancer.
CVD	Cardiovascular diseases.
CLA	Conjugated linoleic acid.
DVS	Direct vat strain.
DNA	Dioxy ribonucleic acid.
EDTA	Ethylene diamine tetra acetic acid.
FAO	Food Agriculture Organization.
FF	Functional foods.
FDA	Food and Drug Administration.
FKB	Fermented kidney beans.
Hb	Hemoglobin.
Hct	Hematocrit.
HDL-C	High density lipoprotein-cholesterol.
HFHC	High fat high cholesterol diet.
HX	Haematoxylin.
IgA	Immunoglobulin type A.