Ain Shams University
Women's College
Biochemistry and Nutrition Department

The Chemopreventive Activity of Cruciferous Vegetables For Suppression of Hypercholesterolemia in Rats

Thesis
Submitted For The Degree of Ph .D Of Philosophy in Science
In Biochemistry and Nutrition

By
Sohear Abd- El-Samea Mohamed
Department of Food Hygiene
National Nutrition Institute

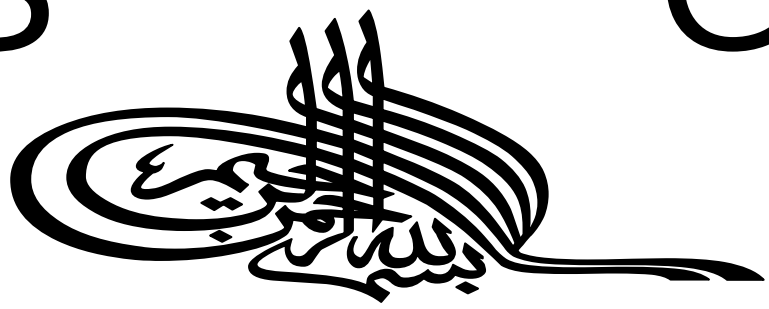
Supervisors
Prof. Dr.
Nagwa Ibrahim Yahia Hassanin
Professor of Nutrition
Biochemistry and Nutrition Department
Women's Collage
Ain Shams University

Prof.Dr.
Salah El-Dein S. Ahmed
Professor of Biochemistry
National Nutrition Institute

Dr. Eman Ali Sadeek Fadel –Alla
Lecturer in Biochemistry and Nutrition
Biochemistry and Nutrition Department

Women's College
Ain Shams University
2005

S G



سبحانك لا علم لنا

إلا ما علمتنا

إنك أنت العليم الحكيم

صدق الله العظيم

¨¢32¢WÂÉdl ÇÉÑu ©

S g

ACKNOWLEGMENT

I would like to express my deepest thanks and gratitude to **Prof. Dr. Nazek Darwish**, professor of Nutrition, Department of Biochemistry and Nutrition, Women's College Ain Shams University, for helpful advice, her valuable assistance and kind supervision.

I would like also to express my greatest thanks and sincere appreciation to **Prof. Dr. Mona Sadek** Prof. of Nutrition and head of Biochemistry and Nutrition Department, Women's College, Ain Shams University for her scientific support, precious supervision and her endless encouragement through this work.

Moreover, special thanks go to **Prof. Dr. Salah El-Dein Ahmed** professor of Biochemistry and **Prof. Dr. Ahmed shaker**, professor of Food Technology, National Nutrition Institute for their valuable help and supervision. Finally I would like to express deep gratitude to **Prof. Dr Ehab Higazy**

Prof. of Food Science and all staff members of Food Science Department, National Nutrition Institute for effort and help to carry out this study.

ABSTRACT

The present study determined the concentration of some trace elements (Fe, Zn, Cu, Se, Cr, Pb and Cd) in some food groups (vegetables, cereals, legumes and milk) by using atomic absorption spectrometry either with burner type in the case of Fe, Zn and Cu detection or equipped with graphite furnace atomic absorption spectrometry in case of the determination of Se, Cr, Pb and Cd. The results were as follows:

1- Vegetable analysis showed that in general leafy vegetables contain the highest levels of Fe, Zn, Se and Cr, also okra as seed pods vegetables contain a high levels of Zn, Se and Cr. Roots and tubers vegetablessuch as potato and yellow carrots reported the highest value of Pb and Cd as well as peas garden from seed pods vegetables.

2- Cereals and legumes analysis showed that wheat as cereals showed the highest levels of Fe, Zn , Se and Cr, while Fe, Zn, Cu, Se and Cr were relatively higher in legumes than in cereals. Pb and Cd levels in cereals and legumes were varied and reflects the environmental pollution, the values still within the permissible levels.

3- Milk analysis showed that the different types of milk either fresh or dried was considered a poor source for tested

trace elements, whereas concentration of Pb and Cd were nearest the minimum range of the permissible levels.

LIST OF CONTENTS

Page

Introduction 1

Review of literature 4

Materials and methods 34

Materials 34

Methods 34

1-Samples preparation 34

2-Methods of analysis 35

2.1. Ashing 35

2.2. Instrumental techniques 36

3-Statistical analysis 37

Results and Discussion 38

1-Levels of tested trace elements in some vegetables 38

1.1 Concentrations of micro trace elements (Fe, Zn, and Cu (ug/g) 38

1.2 Concentrations of ultra trace elements Se and Cr (ng/g). 46

1.3 Concentrations of toxic elements Pb and Cd (ng/g). 51

..... 57

2-Levels of tested trace elements in some cereals and legumes.

Page

2.1. Concentrations of micro trace elements Fe, Zn and Cu (ug/g). 57

2.2. Concentrations of ultra trace elements Se and Cr (ng/g). 63

2.3. Concentrations of toxic elements Pb and Cd (ng/g) 68

3-Levels of tested trace elements in some kinds of milk. 72

3.1. Concentrations of micro trace elements Fe, Zn, and Cu (ug/g). 72

3.2. Concentrations of ultra trace elements Se and Cr (ng/g). 78

3.3. Concentrations of toxic elements Pb and Cd (ng/g) 82

Summary 86

Conclusion 89

References 91

Arabic summary

LIST OF TABLES

Table		Page
Table (1):	Concentration of micro trace elements Fe ,Zn and Cu in tested vegetables on fresh weight basis ug/g (p.p.m).	42
Table (2):	Concentration of ultra trace elements Se and Cr. in tested vegetables on fresh weight basis ng/g (p.p.b).	48
Table (3):	Concentration of toxic elements Pb and Cd in tested vegetables on fresh weight basis ng/g (p.p.b).	54
Table (4):	Concentration of micro trace elements Fe ,Zn and Cu in tested cereals and legumes in fresh weight basis ug/g (p.p.m).	59
Table (5):	Concentration of ultra trace elements Se and Cr in tested cereals and legumes on fresh weight basis ng/g (p.p.b).	65
Table (6):	Concentration of toxic elements Pb and Cd in tested cereals and legume on fresh weight basis ng/g (p.p.b).	69
Table (7):	Concentration of micro trace elements Fe Zn and Cu in tested kinds of milk on fresh weight basis ug/g (p.p.m).	74
Table (8):	Concentration of ultra trace elements Se and Cr in tested kinds of milk on fresh weight basis ng/g (p.p.b).	79

Table (9): Concentration of toxic elements Pb and Cd in tested kinds of milk on fresh weight basis ng/g (p.p.b). 82

LIST OF FIGURES

Figure		Page
Figure (1a):	Concentration of Fe in tested vegetables on fresh weight basis ug/g (p.p.m).	43
Figure (1b):	Concentration of Zn in tested vegetables on fresh weight basis ug/g (p.p.m).	44
Figure (1c):	Concentration of Cu in tested vegetables on fresh weight basis ug/g (p.p.m).	45
Figure (2a):	Concentration of Se in tested vegetables on fresh weight basis ng/g (p.p.b).	49
Figure (2b):	Concentration of Cr. in tested vegetables on fresh weight basis ng/g (p.p.b).	50
Figure (3a):	Concentration of Pb in tested vegetables on fresh weight basis ng/g (p.p.b).	55
Figure (3b):	Concentration of Cd in tested vegetables on fresh weight basis ng/g (p.p.b).	56
Figure (4a):	Concentration of Fe in tested cereals and legumes on fresh weight basis ug/g (p.p.m).	60
Figure (4b):	Concentration of Zn in tested cereals and legumes on fresh weight basis ug/g (p.p.m).	61
Figure (4c):	Concentration of Cu in tested cereals and legumes on fresh weight basis ug/g (p.p.m).	62
Figure (5a):	Concentration of Se in tested cereals and legumes on fresh weight basis ng/g (p.p.b).	66

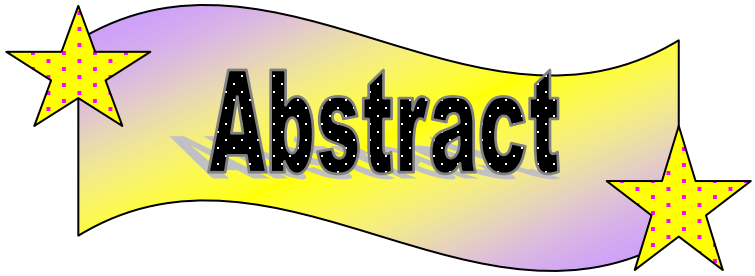
Figure (5b):	Concentration of Cr. in tested cereals and legumes on fresh weight basis ng/g (p.p.b).	67
Figure (6a):	Concentration of Pb in tested cereals and legume on fresh weight basis ng/g (p.p.b).	70
Figure (6b):	Concentration of Cd in tested cereals and legume on fresh weight basis ng/g (p.p.b).	71
Figure (7a):	Concentration of Fe in tested kinds of milk on fresh weight basis ug/g (p.p.m).	75
Figure (7b):	Concentration of Zn in tested kinds of milk on fresh weight basis ug/g (p.p.m).	76
Figure (7c):	Concentration of Cu in tested kinds of milk on fresh weight basis ug/g (p.p.m).	77
Figure (8a):	Concentration of Se in tested kinds of milk on fresh weight basis ng/g (p.p.b).	80
Figure (8b):	Concentration of Cr. in tested kinds of milk on fresh weight basis ng/g (p.p.b).	81
Figure (9a):	Concentration of Pb in tested kinds of milk on fresh weight basis ng/g (p.p.b).	84
Figure (9b):	Concentration of Cd in tested kinds of milk on fresh weight basis ng/g (p.p.b).	85

I would like to express my deepest thanks and gratitude to ***Prof. Dr. Nagwa Ibrahim Yahia Hassanin****, professor of Nutrition,Department of Biochemistry and Nutrition, Women's Collage Ain Shams University, for helpful advice, her valuable assistance , kind supervision, scientific support and her endless encouragement through this work .*

I would like also to express my greatest thanks and sincere appreciation to ***Prof. Dr. Salah El-Dein S. Ahmed*** *Prof. of Biochemistry Department of food hygiene National Nutrition Institute for his precious supervision and valuable help.*

Moreover, special thanks go to ***Dr.Eman Ali Sadeek Fadel-Alla*** *Lecturer of Biochemistry and Nutrition Department of Biochemistry and Nutrition, Women's Collage Ain Shams University for her valuable cooperation and help.*

Finally I would like to express deep gratitude to all staff members of Food Hygiene Department, National Nutrition Institute for effort and help to carry out this study.



The effect of chemopreventive activity of cruciferous vegetables, cabbage (Brassica oleracea L. var. capitata L.) and broccoli (Brassica oleracea L. var. botrytis L.) on plasma, liver and artery lipid concentrations were examined in rats fed diets containing 5g /kg cholesterol and 15% fat (5% corn oil + 10% tallow). Sixty-four adult male albino rats were randomly allocated in-groups of eight rats each to eight diets, which they ate continuously for 42 days.

The diets fed were G1 (Basal diet (BD)+ 15% fat); G2 (BD+ 0.5% cholesterol (CH)+ 15% fat); G3; G5; and G7 (diet of G2+ 7% of either fresh dried or steamed cooked dried cabbage and broccoli respectively); G4; G6 and G8 (diet of G2+ 15% of vegetables substituted for 7%). Fasting blood samples were taken on the day 42 for the determination of antioxidative enzyme activities (GSH-Px & SOD); hemoglobin; plasma, liver and aorta wall total cholesterol. triacylglycerols and phospholipids; plasma HDL-C ; LDL-C ; malondialdhyde (lipid peroxidations) and plasma Zn, Cu and Se. After 42 days, significant elevations ($P<0.01$) were noticed in the values of hemoglobin of rats in group 8 and 6 in comparison with G1 and G2.

By comparing G8 and G6 with G4, it was found that, a significantly increased ($P<0.01$) in GSH-Px of whole blood and GSH-Px of hemolysate in G4 and G8 and between G6 and G8, but not in G4 as compared with G6. Also erythrocyte SOD

activity /gHb was significantly increased using diets 3 and 5 compared with diets 7. While rats of G7 & G8, which given either 7 or 15% of steamed cooked dried broccoli, showed a significant increase in the erythrocyte SOD activity and in erythrocyte SOD/ gHb.

Plasma total cholesterol and LDL-C were affected and significantly decreased by using 15% of steamed cooked dried broccoli (G8), wherein the highest value for the percentage of cholesterol carried by HDL and the lowest value obtained for LDL-C/ HDL-C which is considered as atherogenic factor was obtained using diet 6 followed diet 8. Whilst the lowest value for liver total cholesterol was obtained by G6 followed by G8. No significant difference in plasma and artery triacylglycerols, but liver triacylglycerols was affected and significantly decreased using either diet 6 or 4 compared with diet 2.

A statistical significant decrease in plasma; liver and artery phospholipids in all treated rats. Moreover, there were significant decreases in MDA between all the treated animals as compared to G2.

Thus, we conclude that, daily consumption of these vegetables has a potential property in lowering cholesterol and triacylglycerols concentration in plasma, liver and artery in hypercholesterolemic rats.

List of CONTENTS

	Page
Introduction	**1**
Aim of the Work	**12**
Review of literature:	**13**
Antioxidants effects of Cruciferous Vegetables	**14**
Effects of Cruciferous Vegetables on Immune Functions	**23**
Effects of Cruciferous Vegetables on Lipid Metabolism	**25**
Hypocholesterolimic Effect of Cruciferous Vegetables	**26**
The Anticarcinogenic Effect of Cruciferous Vegetables	**34**
Relationship Between Epidemiological Studies & Cruciferous Vegetables	**46**
Antimicrobial Effect of Cruciferous Vegetables	**51**
A Shelf Life of Cruciferous Vegetables	**60**
Materials and Methods:	**62**
Materials:	**62**
Preparation of Cruciferous Vegetables Samples	**62**
Fresh Dried Cabbage	**62**
Steamed Cooked Dried Cabbage	**62**
Steamed Cooked Dried Broccoli	**63**
Biological Experiments	**64**
Methods:	**65**
Experimental Animals	**66**
Sample Collection:	**67**
Blood	**67**
Tissue	**68**
Biochemical Analysis	**68**
Statistical analysis	**71**
Results	**72**
Discussion	**129**
Conclusion	**148**
Summary	**149**
References	**155**

List of Tables

Table	Page
Table (1): Glucosinolats Commonly Found in Brassica Vegetables	**3**
Table (2): Proximate Analysis Data for Fresh Dried and Steamed Cooked Cabbage and Broccoli	**63**
Table (3): Composition of the High Saturated Fat Diet (g/100g diet)	**64**
Table (4): The Composition of Control and Experimental Diets (g/100g diet)	**66**
Table (5): The Effect of Supplemented Different Concentrations of Cruciferous Vegetables on Initial Weight °Final Weight; Feed intake; Gain in Body Weight (g) & Feed Efficiency Ratio	**74**
Table (6): The Effect of Supplemented Different Concentrations of Cruciferous Vegetables on Relative organs Weight (g %)	**78**
Table (7): The Effect of Supplemented Different Concentrations of Cruciferous Vegetables on the Antioxidant Status of Rats GSH-Px of Whole Blood; GSH-Px of Hemolysate; GSH-Px/gHb & Selenium	**87**
Table (8): The Effect of Supplemented Different Concentration of Cruciferous Vegetables on Erythrocytes SOD Activity, Plasma Zn & Cu-SOD	**95**
Table (9): The Effect of Supplemented Different Concentrations of Cruciferous Vegetables on Plasma Total Cholesterol; LDL-C; HDL-C; Atherogenic index; HDL- C/LDLC & LDL-C/HDL-C	**103**