



Ain Shams University
Women's College for
Art, Science and Education

Effect of some Functional Foods on Induced Cataract in Experimental Animals

**Thesis Submitted for Doctor Philosophy Degree in Science
(Biochemistry and Nutrition)**

By

Hasnaa Hatem Hassan Mohamed

M.Sc. in Biochemistry and Nutrition

2006

Biochemistry and Nutrition Department
Women's college – Ain Shams University

Supervisors

Prof. Dr. Tahany El Sayed Kholeif

Professor of Biochemistry

Biochemistry and Nutrition Department
Women's college – Ain Shams University

Prof. Dr. Mohamed Saad Al-Balkini

Professor of Ophthalmology

Research Institute of Ophthalmology

Prof. Dr. Anhar Mohamed Gomaa Shehab

Professor of Biochemistry and Nutrition

Biochemistry Department

Research Institute of Ophthalmology

Prof. Dr. Fatma Hassan Abd El-Razik

Professor of Nutrition

Biochemistry and Nutrition Department

Women's college – Ain Shams University

Dr. Nora Mohamed Afifi El Sheikh

Assistant Professor of Nutrition

Biochemistry and Nutrition Department

Women's college – Ain Shams University

2012



كلية البنات للآداب والعلوم والتربية

تأثير بعض الأغذية الوظيفية على المياه البيضاء المستحدثة في حيوانات التجارب

رسالة مقدمة من
حسناء حاتم حسن محمد
للحصول على درجة دكتوراه الفلسفة

في
العلوم (الكيمياء الحيوية و التغذية)
قسم الكيمياء الحيوية و التغذية
كلية البنات - جامعة عين شمس

تحت إشراف
أ.د. تهاني السيد خليف
أستاذ الكيمياء الحيوية
قسم الكيمياء الحيوية و التغذية
كلية البنات - جامعة عين شمس

أ.د. أنهار محمد جمعة شهاب
أستاذ الكيمياء الحيوية و التغذية
قسم الكيمياء الحيوية
معهد بحوث أمراض العيون

أ.د. محمد سعد البلاقيني
أستاذ أمراض العيون
قسم أمراض العيون
معهد بحوث أمراض العيون

د. نورا محمد عفيفي الشيخ
أستاذ التغذية المساعد
قسم الكيمياء الحيوية و التغذية
كلية البنات - جامعة عين شمس

أ.د. فاطمة حسن عبد الرزاق
أستاذ التغذية
قسم الكيمياء الحيوية و التغذية
كلية البنات - جامعة عين شمس

رسالة دكتوراه

إسم الطالبة : حسناء حاتم حسن محمد
عنوان الرسالة : تأثير بعض الأغذية الوظيفية على المياه البيضاء
المستحدثة في حيوانات التجارب

لجنة الإشراف

أ.د. تهاني السيد خليف

أستاذ الكيمياء الحيوية
قسم الكيمياء الحيوية والتغذية
كلية البنات - جامعة عين شمس

أ.د. محمد سعد البلقيني

أستاذ أمراض العيون
قسم أمراض العيون
معهد بحوث أمراض العيون

أ.د. أنهار محمد جمعة شهاب

أستاذ الكيمياء الحيوية والتغذية
قسم الكيمياء الحيوية
معهد بحوث أمراض العيون

أ.د. فاطمة حسن عبد الرازق

أستاذ التغذية
قسم الكيمياء الحيوية والتغذية
كلية البنات - جامعة عين شمس

د. نورا محمد عفيفي الشيخ

أستاذ التغذية المساعد
قسم الكيمياء الحيوية والتغذية
كلية البنات - جامعة عين شمس

تاريخ البحث: ٢٠١٢ / / ٢٠١٢

الدراسات العليا:

ختم الإجازة

٢٠١٢ / / ٢٠١٢

أجيزت الرسالة بتاريخ
٢٠١٢ / / ٢٠١٢

موافقة مجلس الكلية

٢٠١٢ / / ٢٠١٢

موافقة مجلس الجامعة
٢٠١٢ / / ٢٠١٢

عنوان الرسالة

تأثير بعض الاغذية الوظيفية على المياه البيضاء المستحدثة في حيوانات التجارب

إسم الطالبة : حسناء حاتم حسن محمد

الدرجة العلمية : درجة دكتور الفلسفة في العلوم (كيمياء حيوية و تغذية)

القسم : قسم الكيمياء الحيوية و التغذية

الكلية : كلية البناء

الجامعة : جامعة عين شمس

سنة التخرج : ١٩٩٧

سنة المنح : ٢٠١٢

Effect of some Functional Foods on Induced Cataract in Experimental Animals

Hasnaa Hatem Hassan Mohamed. Thesis Submitted for Ph.D. Degree in Science in "Biochemistry and Nutrition".

Abstract

Functional foods are foods that, by virtue of physiologically active components, provide health benefits beyond basic nutrition.. The object of the study is to examine the effect of dietary intake of garlic, onion, broccoli and soybean powders on preventing cataract formation. Total phenolic compounds in garlic, onion, broccoli and soybean powders were determined. Antioxidant and apoptotic status in addition to lipids profile and their relation to cataract were also investigated. Sixty five Wister rat pups were divided into ten groups. Group 1 (normal control, n=6) received basal diet; group 2 (cataract, n=8) received a single subcutaneous (s.c.) injection of sodium selenite (Na_2SeO_3) ($30\mu\text{mol}/\text{kg}$ body weight) and fed on basal diet; group 3 (normal, fed on garlic, n=6) received basal diet containing 5% garlic powder; group 4 (cataract, fed on garlic, n=7) received s.c. injection of Na_2SeO_3 and fed on basal diet containing 5% garlic powder, group 5 (normal, fed on onion, n=6) received basal diet containing 10% onion powder; group 6 (cataract, fed on onion, n=6) received s.c. injection of Na_2SeO_3 and fed on basal diet containing 10% onion powder; group 7 (normal, fed on broccoli, n=7) received basal diet containing 10% broccoli powder; group 8 (cataract, fed on broccoli, n=6) received s.c. injection of Na_2SeO_3 and fed on basal diet containing 10% broccoli powder; group 9 (normal, fed on soybean, n=6) received basal diet containing 25% soybean powder and group 10 (cataract, fed on soybean, n=7) received s.c. injection of Na_2SeO_3 and fed on basal diet containing 25% soybean powder.

At the end of the experiment (two months) catalase (CAT), superoxide dismutase (SOD) activities, total antioxidant capacity (TAO), reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NO) were assessed in both blood and lenses. Fas ligand (FAS-L) as apoptotic marker was also assessed in the blood and lenses. The levels of serum lipid profile, serum glucose and blood

hemoglobin were determined in rats' blood. The crystalline lens protein patterns on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were identified and analyzed by computerized program.

Results of slit-lamp examination of both eyes of each rat revealed no lenticular opacification (cataract stage 0) in all eyes of groups 1,3,5,7 and 9. Complete lens opacification (stage 4) was noticed in the group 2 (100%), while mild lenticular opacification (cataract stage1) was noticed in 14.3%, 16.7%, 16.7% and 28.7% of rats in the groups treated with garlic, onion, broccoli and soybean, respectively. The mean activities of the antioxidant enzymes CAT and SOD and levels of TAO and GSH were significantly lower in blood and lenses of cataractous group than normal control group. Conversely, the mean levels of MDA, NO and FAS-L were significantly higher in blood and lenses of cataractous rats than normal control. Serum glucose was significantly higher in group 2 than in group 1, while blood hemoglobin didn't show any significant difference. In addition, the mean levels of total lipids (TL), triacylglycerols (TG), total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) were significantly higher in selenite induced cataract group than in those of normal control. However, 5% garlic, 10% onion, 10% broccoli and 25% soybean powders significantly reversed the effect of sodium selenite on cataractous rats and improved the previous parameters. Also SDS-PAGE showed reduction of the soluble lens protein peaks and intensities in cataract group, while the peaks numbers and intensities of cataract groups fed on garlic, onion, broccoli and soybean were recovered in variable degrees. In conclusion, the results of this study suggested that consumption of diet containing 5% garlic, 10% onion, 10% broccoli and 25% soybean powders can prevent or retard selenite-induced cataract in Wistar rats. Also, they improved antioxidant status, reduced oxidative stress and apoptosis; and improved lipid profile in both normal and cataractous rats.

Key words: functional foods- garlic- onion- broccoli- soybean- selenite-induced cataract- phenolic compounds- oxidative stress- Fas-L- nitric oxide- lipid profile.

List of Contents

ABSTRACT	1
INTRODUCTION	3
AIM OF THE WORK	8
REVIEW OF LITERATURE	
I- The Lens	9
Lens Physiology	9
Lens Proteins	11
Lens Metabolism	13
II- Cataract	14
Definition and Symptoms	14
Types of cataract	15
Factors Causing Cataract	16
III- Oxidative stress	18
Reactive oxygen species	18
Antioxidant Defense Systems	21
Biochemical Effects of Nitric Oxide	23
Cataract and Oxidative Stress	25
Sodium Selenite Induced Cataract	28
VI- Apoptosis and Eye	31
V- Functional Foods	35
Examples of Functional Foods	40
I. Garlic	40
2. Onion	42
3. Broccoli	44
4. Soybean	46
MATERIALS AND METHODS	
Materials	49
Chemicals	49
Diet	49
Tested Functional Foods	49
Experimental Animals	49
Methods	50
I- Chemical Analysis of Phenolic Compounds by HPLC for Garlic, Onion, Broccoli and Soybean powder	50

II- Experimental Design	51
III- Induction of selenite cataract	52
IV- Ophthalmic Examination.....	52
V- Blood Sampling	53
VI- Biochemical Analyses	54
A-Whole Blood Analysis	54
Determination of Blood Hemoglobin.....	54
Determination of Superoxide Dismutase Activity (SOD).....	54
Determination of Reduced Glutathione (GSH).	56
B- Plasma Analysis	57
Determination of Catalase Activity (CAT).....	57
C- Serum Analysis.....	58
Determination of Blood Glucose.....	58
Determination of Total Antioxidant Capacity (TAO).....	59
Determination of Malondialdehyde (MDA).....	60
Determination of Nitric Oxide (NO).....	61
Determination of Fas Ligand (Fas-L).....	62
Determination of Total Lipids (TL).....	64
Determination of Triacylglycerols (TAG).....	65
Determination of Total Cholesterol (TC).....	67
Determination of High Density Lipoprotein-Cholesterol (HDL-C).....	68
Determination of Low Density Lipoprotein-Cholesterol (LDL-C).....	69
D- Lens Analysis	69
Determination of Total Protein in Lens (TP).....	69
VII- Lens Proteins Identification by SDS-PAGE	71
VIII- Statistical Analysis	72
 RESULTS	
Slit Lamp Examination and Degree of Lens Opacification..	73
Results of Liquid Chromatography	77
Results of Biochemical Assessment	80
Results of SDS-PAGE	124
 DISCUSSION	129
 SUMMARY	174
 CONCLUSION AND RECOMMENDATIONS	180
 REFERENCES	181

List Of tables

Table number	Title	Page
1	Grading of cataract on the basis of slit-lamp examination	76
2	Phenolic compounds in garlic and onion by high performance liquid chromatography (HPLC).	77
3	Phenolic compounds in garlic and onion by high performance liquid chromatography (HPLC).	78
4-a	Mean±S.E and change% for catalase activity (CAT), superoxide dismutase activity (SOD), total antioxidants (TAO), reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and fas ligand (fas-L) for normal and cataract groups with and without garlic administration.	81
4-b	Means±S.E. and change% for serum total lipids (TL), triacylglycerols (TAG), total cholesterol (TC) , high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), glucose and blood hemoglobin (Hb) for normal and cataract groups with and without garlic administration.	85
5-a	Mean±S.E. and change% for catalase activity (CAT), superoxide dismutase activity (SOD), total antioxidants (TAO), reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and fas ligand (fas-L) for normal and cataract groups with and without onion administration.	89
5-b	Mean±S.E. and change% for serum total lipids (TL), triacylglycerols (TAG), total cholesterol (TC) , high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), glucose and blood hemoglobin (Hb) for control and cataract groups with and without onion administration.	93

6-a	Mean±S.E. and change% for catalase activity (CAT), superoxide dismutase activity (SOD), total antioxidants (TAO), reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and fas ligand (fas-L) for normal and cataract groups with and without broccoli administration.	97
6-b	Mean±S.E. and change% of serum total lipids (TL), triacylglycerols (TAG), total cholesterol (TC) , high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), glucose and blood hemoglobin (Hb) for normal and cataract groups with and without broccoli administration.	101
7-a	Mean±S.E. and change% for catalase activity (CAT), superoxide dismutase activity (SOD), total antioxidants (TAO), reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and fas ligand (fas-L) for normal control and cataract groups with and without soybean administration.	105
7-b	Mean±S.E. and change% for serum total lipids (TL), triacylglycerols (TAG), total cholesterol (TC) , high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), glucose and blood hemoglobin (Hb) for normal and cataract groups with and without soybean administration.	109
8	Levels of total protein (TP), catalase activity (CAT), superoxide dismutase activity (SOD), total antioxidants (TAO), , reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and fas ligand (fas-L) in rats lenses of normal and cataract groups with and without treatment.	116

List of Figures

Figure number	Title	Page
1	Cross-section of a mammalian eye showing the position of the lens.	9
2	Diagrammatic representation of the mammalian lens	11
3	Illustrations of the types of cataracts and lens anatomy.	16
4	Mechanism of selenite nuclear cataract formation.	30
5	Chemical structure of flavonoids	39
6	Biosynthetic pathway of thiosulfinate.	41
7	Sapogenins found in garlic and onion.	42
8	Chemical structure of sulforaphane.	45
9	Structures of genistein (A) and daidzein (B).	48
10	Standard curve of Nitrite	62
11	Fas-L standard curve ranging from 62.5 to 2000 pg/ml	64
12	standard curve of total lens protein	70
13	Eyes of cataract group rats after 10 days of starting the experiment.	73
14	Eyes of normal control group rats after 10 days of sodium selenite injection	73
15	Eyes of cataract treated groups after 10 days of sodium selenite injection (a) Cataract group fed on garlic. (b) Cataract group fed on onion (c) Cataract group fed on broccoli (d) Cataract group fed on soybean	74
16	Eyes of cataract group at the end of experiment.	74
17	Eyes of normal control group at the end of experiment.	75

18	Eyes of cataract treated groups at the end of experiment. (a) Cataract group fed on garlic. (b) Cataract group fed on onion (c) Cataract group fed on broccoli (d) Cataract group fed on soybean	75
19-a	Catalase activity (CAT U/L/10), superoxide dismutase activity (SOD U/ml), total antioxidants (TAO mmol/L/10), reduced glutathione (GSH mg/dl), malondialdehyde (MDA nmol/ml×10), nitric oxide (NO µmol/L×10), and fas ligand (fas-L Pg/ml/10) for normal control and cataract groups with and without garlic administration.	82
19-b	Percentage change from normal control group for catalase activity, superoxide dismutase activity, total antioxidants, reduced glutathione, malondialdehyde, nitric oxide and fas ligand for normal group fed on garlic, cataract group and cataract group fed on garlic.	83
19-c	Total lipids (TL mg/dl/10), triacylglycerols (TAG mg/dl), total cholesterol (TC mg/dl) , high density lipoprotein-cholesterol (HDL-C mg/dl), low density lipoprotein-cholesterol (LDL-C mg/dl), glucose and blood hemoglobin (Hb mg/dl) for normal control and cataract groups with and without garlic administration.	86
19-d	Percentage change from normal control group for serum total lipids, triacylglycerols, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, glucose and blood hemoglobin for normal group fed on garlic, cataract group and cataract group fed on garlic.	87

20-a	Catalase activity (CAT U/L/10), superoxide dismutase activity (SOD U/ml), total antioxidants (TAO mmol/L/10), reduced glutathione (GSH mg/dl), malondialdehyde (MDA nmol/ml×10), nitric oxide (NO μ mol/L×10), and fas ligand (fas-L Pg/ml/10) for normal control and cataract groups with and without onion administration.	90
20-b	Percentage change from normal control group for catalase activity, superoxide dismutase activity, total antioxidants, reduced glutathione, malondialdehyde, nitric oxide and fas ligand for normal group fed on onion, cataract group and cataract group fed on onion.	91
20-c	Total lipids (TL mg/dl/10), triacylglycerols (TAG mg/dl), total cholesterol (TC mg/dl) , high density lipoprotein-cholesterol (HDL-C mg/dl), low density lipoprotein-cholesterol (LDL-C mg/dl), glucose and blood hemoglobin (Hb mg/dl) for normal control and cataract groups with and without onion administration.	94
20-d	Percentage change from normal control group for serum total lipids, triacylglycerols, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, glucose and blood hemoglobin for normal group fed on onion, cataract group and cataract group fed on onion.	95
21-a	Catalase activity (CAT U/L/10), superoxide dismutase activity (SOD U/ml), total antioxidants (TAO mmol/L/10), reduced glutathione (GSH mg/dl), malondialdehyde (MDA nmol/ml×10), nitric oxide (NO μ mol/L×10), and fas ligand (fas-L	98

	Pg/ml/10) for normal control and cataract groups with and without broccoli administration.	
21-b	Percentage change from normal control group for catalase activity, superoxide dismutase activity, total antioxidants, reduced glutathione, malondialdehyde, nitric oxide and fas ligand for normal group fed on broccoli, cataract group and cataract group fed on broccoli.	99
21-c	Total lipids (TL mg/dl/10), triacylglycerols (TAG mg/dl), total cholesterol (TC mg/dl) , high density lipoprotein-cholesterol (HDL-C mg/dl), low density lipoprotein-cholesterol (LDL-C mg/dl), glucose and blood hemoglobin (Hb mg/dl) for normal control and cataract groups with and without broccoli administration.	102
21-d	Percentage change from normal control for serum total lipids, triacylglycerols, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, glucose and blood hemoglobin for normal group fed on broccoli, cataract group and cataract group fed on broccoli.	103
22-a	Catalase activity (CAT U/L/10), superoxide dismutase activity (SOD U/ml), total antioxidants (TAO mmol/L/10), reduced glutathione (GSH mg/dl), malondialdehyde (MDA nmol/ml×10), nitric oxide (NO µmol/L×10), and fas ligand (fas-L Pg/ml/10) for normal control and cataract groups with and without soybean administration.	106
22-b	Percentage change from normal control for catalase activity, superoxide dismutase activity, total antioxidants, reduced glutathione, malondialdehyde, nitric oxide	107

	and fas ligand for normal group fed on soybean, cataract group and cataract group fed on soybean.	
22-c	Total lipids (TL mg/dl/10), triacylglycerols (TAG mg/dl), total cholesterol (TC mg/dl) , high density lipoprotein-cholesterol (HDL-C mg/dl), low density lipoprotein-cholesterol (LDL-C mg/dl), glucose and blood hemoglobin (Hb mg/dl) for normal control and cataract groups with and without soybean administration.	110
22-d	Percentage change from normal control for serum total lipids, triacylglycerols, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, glucose and blood hemoglobin for normal group fed on soybean, cataract group and cataract group fed on soybean.	111
23-a	Percentage change from cataract for catalase activity, superoxide dismutase activity, total antioxidants, reduced glutathione, malondialdehyde, nitric oxide, and fas ligand for cataract groups fed on garlic, onion, broccoli and soybean.	113
23-b	Percentage change from cataract for serum total lipids, triacylglycerols, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, glucose and blood hemoglobin for cataract groups fed on garlic, onion, broccoli and soybean.	114
24	Percentage change from normal for lens total protein (TP mg/g lens), catalase (CAT U/ mg lens protein), superoxide dismutase (SOD U/ mg lens protein), total antioxidants (TAO mmol/ mg lens protein), reduced glutathione (GSH μ g/ mg lens protein),	117