بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأفلام قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٢٥ مئوية ورطوبة نسبية من ٢٠-٤٠%

To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%

بعض الوثائق الأصلية تالفه

بالرسالة صفحات
لم ترد بالاصل

ANTIOXIDANT AND ANTIATHEROGENIC ROLE OF SOME VITAMINS

By

SAHAR MOSTAFA KAMEL SALEM

B.Sc. Agric. (Biochemistry), Cairo Univ.1992.

M.Sc. Agric. (Biochemistry), Cairo Univ. 1997.

THESIS

Submitted for Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

Agricultural Biochemistry

TO

Biochemistry Department

Faculty of Agriculture

Cairo University

2002

بِسمِ اللهِ الرَّحمَنِ الرَّحيمِ

رَبِّ أَوزِعنِى أَن أَشكُرَ نِعمَتَكَ
الَّتِى أَنعَمتَ عَلَىَّ وَعَلَىٰ وَالِدَىَّ
وَأَنْ أَعمَلَ صَالِحاً تَرضَاهُ
وَأَدخِلنِى بِرَحمَتِكَ فِى عِبَادِكَ
الصَّالِحِينَ

صَدَقَ اللهُ العَظِيم.

مِنَ الآيه (١٩) سورة النَمل

APPROVAL SHEET

ANTIOXIDANT AND ANTIATHEROGENIC ROLE OF SOME VITAMINS

By

SAHAR MOSTAFA KAMEL SALEM

M.Sc. Agric. (Biochemistry)

Cairo Univ. 1997.

This thesis for Ph.D. degree has been approved by:

Prof. Dr. ....

Prof. Dr. .....

Prof. Dr. .....

Committee in charge

Date: 10/ 12/ 2002

Name of Candidate: Sahar Mostafa Kamel Salem **Degree:** Ph.D.
Title of Thesis: Antioxidant and antiatherogenic role of some vitamins
Supervisors: Prof..Dr. Mohy El-Din Ali Osman
Prof. Dr. Ahmed E. Basyony
Department: Biochemistry
Branch: Biochemistry **Approval:** / /

ABSTRACT

The involvement of lipids in early development of arteriosclerosis has been confirmed. Association of antioxidants with n-3 polyunsaturated fatty acids could prevent lipid peroxidation and increase the antiatherogenic effects of n-3 polyunsaturated fatty acids.

This study was planed to investigate the *in vitro* susceptibility of low-density lipoprotein (LDL) cholesterol to oxidation with or without Cu^{2+} or Fe^{2+} and the antioxidant protection of vitamins C and E. Also to examine the hypocholesterolemic, antiatherogenic and antioxidant effects of supplementation with vitamins C and/or E *in vivo*.

A. *In vitro* study on vitamins C and/or E activities as antioxidant.

Plasma LDL cholesterol of normal male albino rats was oxidized with or without exposure to Cu^{+2} or Fe^{+2} to register Δ abs at 234nm as indication for accumulation of conjugated dienes. The obtained results showed that: 1-The presence of metal ions of Cu^{+2} or Fe^{+2} have a prooxidant effect.

2- The prooxidant effect of Cu^{+2} or Fe^{+2} was strongly inhibited by vitamin C compared to the absence of metal ions where vitamin C + E was more effective as antioxidant than vitamins C or E alone.

B. Effect of vitamins C and/or E on rats fed different sources of lipids:

Tow experiments were conducted to study the effect of lipid source, i.e. sunflower oil as source of polyunsaturated fatty acids and sheep tail fat as source of saturated fatty acids as well as the effect of vitamins C and/or E supplementation as antioxidant and antiatherogenic agents. The results revealed that:

A) Relative body weight gain and organs weight percentage.

1-The lipid source has no significant effect on the relative body weight gain % in normal as well as the hypercholestrolemic groups.

2- No significant difference in relative liver weight % of normal rats fed sunflower oil or sheep tail fat was observed. Meanwhile relative liver weight % increased significantly in of hypercholesterolemic untreated rats fed sheep tail fat compared with those fed sunflower oil.

3- Generally no significant differences in relative heart, spleen and kidney weight % due to lipid source or vitamin supplementation were found.

B) Plasma lipid profile

1-Feeding sheep tail fat increased plasma total cholesterol significantly compared with sunflower oil fed groups. Supplementation of vitamin C and/or E to hypercholesterolemic rats fed sunflower oil or sheep tail fat reduced plasma total cholesterol level significantly compared to hypercholesterolemic groups. Supplementation with vitamin C +E to hypercholesterolemic rats fed sunflower oil increased plasma HDL significantly to a level of normal control. Meanwhile supplementation with vitamin C to hypercholesterolemic rats fed sheep tail fat

M. A. Osman

increased HDL nearly to normal control level.

3-Feeding sheep tail fat to normal control group significantly increased plasma VLDL+LDL cholesterol than that fed sunflower oil. The effect of supplementation with different doses of vitamins C and/or E to hypercholesterolemic groups followed similar trend of total cholesterol.

4-The atherogenic index in sunflower oil fed rats was significantly lower than those fed sheep tail fat in normal and hypercholesterolemic untreated groups. Supplementation with vitamin C+ 200 mg vitamin E or C+ 75 mg E in sunflower oil fed rats decreased the atherogenic index significantly compared to the hypercholesterolemic rats. Supplementation with vitamins C and/or E to hypercholesterolemic rats fed sheep tail fat significantly lowered atherogenic index.

5-Plasma triglycerides after feeding hypercholesterolemic diet containing sunflower oil increased significantly, while feeding sheep tail fat increased triglycerides to non significant level compared with normal control.

6- Supplementation of vitamin C and/or E reduced liver total cholesterol level of hypercholesterolemic rats fed sunflower oil or sheep tail fat compared to untreated hypercholesterolemic rats. However feeding sheep tail fat increased liver triglycerides significantly compared with those fed sunflower oil. Feeding cholesterol (0.5%) significantly increased liver triglycerides in rats fed either sunflower oil or sheep tail fat when compared to their respective controls. Supplementation with vitamin C+75 mg vitamin E were the lowest in liver cholesterol and triglycerides levels in all groups fed sunflower oil or sheep tail fat.

7- Conjougated dienes formation was higher in sunflower fed groups than sheep tail fat fed groups. Feeding hypercholesterolemic diet to rats fed sunflower oil showed significant increase in conjugated dienes compared with normal control. However supplementation with vitamin C and/or E reduced this increase to the normal level.

8-Thiobarbeturic acid reactant substances (TBARS) were higher in rats fed sunflower oil than those fed sheep tail fat. Supplementation with vitamin C +200mg vitamin E was the only group reached the level of normal group. Hypercholesterolemic rats fed sheep tail fat the group supplemented with vitamin C +75 mg vitamin E was the best treatment in this respect.

9-Vitamin E level in plasma of all groups of rats fed sheep tail fat was higher than those fed sunflower oil even those groups supplemented or non supplemented with vitamin E at the end of the experimental period.

c-The histopathological effect of vitamins C and/or E showed that feeding hypercholesterolemic diet and sunflower oil over the 60 days showed vaculation of hepatocytes with focal mononuclear cell aggregation and aorta of these rats showed atherosclerosis. The treatment with vitamin C + 200 mg vitamin E inhibited this reverse effect. Feeding hypercholesterolemic diet and sheep tail fat resulted in vaculation of hepatocytes with foamy cytoplasm and narrowing of hepatic sinusoid. However aorta revealed atherosclerosis. The treatment with vitamin C + 75 mg vitamin E inhibited this effect.

M. A. O Sman

List of content

List of content Page No

Introduction **1**

1.REVIEW OF LITERATURE **3**

1.1. *In vitro* studies: Antioxidant activity of ascorbic acid and α-tocopherol in plasma peroxidation..... 3

1.2. The role of vitamin C and vitamin E as antiatherogenic agents (*in vivo*)..... 10 10

1.2.1. The anti-atherogenic properties of vitamin C..... 17

1.2.2. The anti-atherogenic properties of vitamin E..... 29

1.2.3. The interaction between vitamin C , E and atherogenesis.....

2. MATERIALS AND METHODS..... **37**

2.1.Materials..... 37

2.2.1. *In vitro* study on the activity of vitamin C and vitamin E as antioxidants in plasma..... 37

2.2.2. Effect of different concentrations of vitamin C..... 39

2.3. *In vivo* study on the effect of lipid source, ascorbic acid and tocopherol on hypercholesterolemic rats..... 39

2.3.1. Experimental animals..... 39

2.3.2. Blood sampling..... 40

2.3.3. Organs..... 40

2.3.4. Plasma lipid assay:..... 43

2.3.4.1. Determination of total cholesterol..... 43

2.3.4.2. Determination of high-density lipoprotein cholesterol (HDL). 43

2.3.4.3. Determination of triglycerides..... 43

2.3.4.4. Determination of VLDL+LDL cholesterol..... 43

2.3.4.5. Atherogenic index..... 43
2.3.4.6.Determination of plasma thiobarbeturic acid reactive substances..... 44
2.3.4.7. HPLC determination of vitamin E..... 44
2.3.4.8. GLC characterization and determination of fatty acids 45
2.3.5. Liver assay..... 46
2.3.5.1. Determination of total cholesterol and triglycerides 46
2.3.6. Histopathological examination..... 46
2.4. Statistical analysis..... 46
3.RESULTS AND DISCUSSION..... 47
3.1. *In vitro* study on the activity of vitamins C and/or E as antioxidants in plasma..... 47
3.2. *In vivo* studies: Effect of vitamin C and/or E on rats fed different sources of lipids:..... 54
3.2.1 Relative body weight gain and organs weight percentage..... 54
3.2.1. 1. Relative body weight gain percentage..... 54
3.2.1.2. Relative organs weight percentage 59
3.2.2. Lipid profile..... 61
3.2.2.1. Plasma total cholesterol..... 61
3.2.2.2. Plasma HDL-cholesterol..... 67
3.2.2.3. Plasma LDL-cholesterol..... 73
3.2.2.4. Atherogenic index..... 79
3.2.2.5. Plasma triglycerides 85
3.2.2.6. Liver cholesterol..... 90
3.2.2.7. Liver triglycerides..... 90
3.2.2.8. Plasma conjugated dienes..... 94
3.2.2.9. Plasma thiobarbeturic acid reactant substances (TBARS)..... 96
3.2.2.10. HPLC analysis of plasma vitamin E..... 97

3.2.3. The histopathological effect of lipid source, vitamins C and/or E ... 99

Conclusion..... 111

4.Summary..... 112

5.References..... 117

6.Arabic summary

ACKNOWLEDGEMENT

First and forever, ultimate thanks to ALLAH the most beneficent and merciful, and without his aid this work could not be done

The author would like to express her deepest gratitude and appreciation to **Prof. Dr. Mohy El-Din Ali Osman,** *Professor of Biochemistry Department, Faculty of Agriculture, Cairo University, for his closed supervision, encouragement, guidance, valuable advice and his unlimited help during preparation and unfailing help in writing the manuscript.*

The author is also thankful to **Prof. Dr. Ahmed E. Basyony,** *Professor of Biochemistry & Deputy Director, Environmental Affairs and Industry Modernization, FTRI, Agricultural Research Center for his supervision and valuable help.*

I also gratefully acknowledge the help given by **Dr. Ahmed H. Osman** *lecturer of pathology. Faculty of Vet. Med., Cairo University, for his help in the histopatholgical work.*

I am also, thankful to **Prof. Dr. Amal Hassanein Mahmoud** *Professor of Special Food & Nutrition, FTRI, Agricultural Research Center*

I wish to express my thanks to **Prof. Dr. Saeb Abd El Monem Hafez** *Professor of Special Food & Nutrition, FTRI, Agricultural Research Center.*

Special thanks to my husband **Dr. Farag Ali Saleh** *for his unlimited help and encouragement through the course of this work.*

I would like to extend my deepest thanks to ***my father, mother, sisters and brother*** *for their unlimited help.*

Last but not least I would like to thank all members of Special Food & Nutrition, FTRI, Agricultural Research Center and all staff members of Biochemistry Department, Faculty of Agriculture, Cairo University.

Introduction