



Original / *Investigación animal*

Prolonged flaxseed flour intake decreased the thickness of the aorta and modulates some modifiable risk factors related to cardiovascular disease in rats

Ludmila Ferreira Medeiros de França Cardozo^{1,2}, Gabriela Câmara Vicente^{1,3}, Lívia Hipólito Cardozo Brant^{1,3}, Denise Mafra², Maurício Alves Chagas⁴ and Gilson Teles Boaventura^{1,3}

¹Laboratory of Experimental Nutrition (LabNE). College of Nutrition. Federal Fluminense University (UFF). ²Graduate Programme in Cardiovascular Sciences. Federal Fluminense University (UFF). ³Graduate Programme in Medical Sciences. Federal Fluminense University (UFF). ⁴Laboratory of Cellular and Extracellular Biomorphology. Federal Fluminense University (UFF). Brazil.

Abstract

Cardiovascular diseases are a major public health problem. Different risk factors have been recognized as the main causes of the development and progression of cardiovascular diseases. Flaxseed is a source of dietary fiber, lignans, and alpha-linolenic acid. The aim of this study was to evaluate the effects of prolonged supplementation with flaxseed flour as preventive therapy on cardiovascular risk parameters in healthy Wistar rats.

Material and Methods: Female Wistar rats were divided into two groups after giving birth and during lactation period: the control group was fed with diet based on casein and the flaxseed group was fed with diet based on casein containing 25% of flaxseed flour. At weaning, 10 male offspring from each group continued to receive the same diets from their mothers during 250 days. The body weight, visceral fat mass, cholesterol, triglycerides, HDL, VLDL, glucose and thickness of the aorta were analyzed.

Results: The body weight, visceral fat mass, cholesterol, triglycerides, HDL, VLDL, glucose and thickness of the aorta values were statistically lower when compared to control group.

Conclusion: The data suggest that flaxseed flour supplementation in healthy wistar rats for a prolonged period may decrease the thickness of the aorta and may be used as a preventive measure in modulating some modifiable risk factors related to cardiovascular disease.

(Nutr Hosp. 2014;29:376-381)

DOI:10.3305/nh.2014.29.2.7020

Key words: *Flaxseed. Rats. Cardiovascular disease. Lipid profile.*

Correspondence: Ludmila Ferreira Medeiros de França Cardozo. Universidade Federal Fluminense. Laboratório de Nutrição Experimental. Departamento de Nutrição e Dietética. Faculdade de Nutrição. Rua Mários Santos Braga, 30-5º andar. 24020-140 Niterói. Brasil. E-mail: ludmila.cardozo@gmail.com

Recibido: 5-VII-2013.
1.ª Revisión: 9-X-2013.
Aceptado: 18-X-2013.

LA INGESTA PROLONGADA DE HARINA DE LINAZA DISMINUYÓ EL ESPESOR DE LA AORTA Y MODULA ALGUNOS FACTORES DE RIESGO MODIFICABLES RELACIONADOS CON LA ENFERMEDAD CARDIOVASCULAR EN RATAS

Resumen

Las enfermedades cardiovasculares son un problema de salud pública. Diferentes factores de riesgo han sido reconocidos como las principales causas del desarrollo y la progresión de las enfermedades cardiovasculares. La linaza es una fuente de fibra dietética, lignanos, y el ácido alfa-linolénico. El objetivo de este estudio fue evaluar los efectos de la suplementación prolongada con harina de linaza como tratamiento preventivo de los parámetros de riesgo cardiovascular en ratas Wistar saludables.

Material y métodos: Ratas hembras Wistar fueron divididas en dos grupos después de dar a luz y durante el período de lactancia: el grupo de control fue alimentado con una dieta basada en caseína y el grupo de la linaza se alimentó con una dieta basada en caseína que contiene 25% de harina de linaza. Al destete, 10 ratas macho de la camada de cada grupo siguió recibiendo las mismas dietas de sus madres durante 250 días. El peso corporal, la masa grasa visceral, colesterol, triglicéridos, HDL, VLDL, la glucosa y el grosor de la aorta fueron analizados.

Resultados: El peso corporal, la masa grasa visceral, colesterol, triglicéridos, HDL, VLDL, la glucosa y el grosor de la aorta esos valores fueron estadísticamente menor en comparación con el grupo control.

Conclusión: Los datos sugieren que la suplementación con harina de linaza en ratas Wistar saludables durante un período prolongado puede disminuir el espesor de la aorta y puede ser utilizado como una medida preventiva en la modulación de algunos factores de riesgo modificables relacionados con la enfermedad cardiovascular.

(Nutr Hosp. 2014;29:376-381)

DOI:10.3305/nh.2014.29.2.7020

Palabras clave: *Linaza. Ratonés. Enfermedad cardiovascular. Perfil lipídico.*

List abbreviation

ALA: alpha-linolenic acid.
CG: Control Group.
FG: Flaxseed Group.
HDL: high density lipoprotein.
LA: linoleic acid.
LabNE: Experimental Nutrition Laboratory.
LDL: low density lipoprotein.
SDG: secoisolariciresinol diglycoside.
UFF: Federal Fluminense University.
VLDL: very low density lipoprotein.

Introduction

Cardiovascular diseases are a major public health problem and are responsible for 17.5 million deaths of people in the world, with 80% of these deaths occur in developing countries. It is estimated that in 2030 about 23.6 million people will die from this disease¹.

Different risk factors have been recognized as the main causes of the development and progression of cardiovascular diseases. They are divided into modifiable risk factors such as hypercholesterolemia, overweight or obesity, hypertension, sedentary lifestyle, excessive alcohol intake, smoking and diabetes; and not modifiable, such as gender and heredity or a positive family history and age².

Aging affects cardiovascular health, because with the increasing age there is an enlargement of the lumen area, wall thickening and loss of elasticity, thereby promoting changes in cardiac structure and function, and these changes were most evident in the aorta and major arteries³. The vascular stiffening results in hyperplasia and elastin breakdown in the middle layer, responsible for the thickening of the arterial wall. This can contribute to endothelial dysfunction, cardiovascular events such as hypertension and atherosclerosis⁴.

With respect to modifiable risk factors, researcher has observed that the control of these factors is an effective way to reduce the risk of these diseases⁵. As an example, the reduction in saturated fat intake and/or supplementation of polyunsaturated fats.

In this context, the consumption of functional foods is growing by population, and flaxseed (*Linum usitatissimum*) has been very popular due to its health benefits. This oilseed is a source of dietary fiber, lignans, protein, and contain 41% lipid, 50-55% being composed of alpha-linolenic acid (ALA), 15-18% at linoleic acid (LA) and 18% by monounsaturated fatty acids omega-9⁶.

Because of its composition, the seed has proved able to act as an effective factor of protection and reducing some of these cardiovascular risk factors such as glucose lowering⁷, improved lipid profile⁸, weight reduction and obesity⁹, and reduction of visceral fat mass¹⁰.

Thus, the objective of this study was to evaluate the effects of prolonged consumption of flaxseed as

preventive therapy on cardiovascular risk parameters in healthy Wistar rats with 250 days of life.

Materials and methods

Experimental design

Twenty female Wistar rats from the colony of the Experimental Nutrition Laboratory (LabNE) from Federal Fluminense University (UFF) with 90 days old, mated at a ratio of 3 females to 1 male, receiving commercial diet (23% of protein, Nuvilab®, Nuvital Ltda., Paraná State, Brazil).

Female Wistar rats were randomly divided into two groups after giving birth. During the lactation period the Control Group (CG, n = 10) was fed with a diet based on casein containing approximately 18% of protein, and Flaxseed Group (FG, n = 10) with a diet based on casein, containing approximately 18% of protein with addition of 25% of flaxseed flour.

At weaning, 10 male offspring from each group continued on their origin groups, however to receiving diets with approximately 11% of protein (AIN-93M) until 250 days old when they were killed. The weight and diet consumption were registered three times a week. All animals were kept in an animal house with controlled temperature (21-23°C) and light/dark cycle (12/12 hours), receiving water and food *ad libitum*.

This research project was approved by the Animal Research Ethics Committee of the Faculty Office of Research and Postgraduate Studies at the Federal Fluminense University, Niterói, Brazil, under n. 00105-09. All procedures followed the norms of the National Research Council (US) Institute for Laboratory Animal Research¹¹.

Experimental diets

The diets used during lactation were prepared in the Experimental Nutrition Laboratory of the UFF according to the American Institute of Nutrition (AIN 93G) recommendation for rodent diets (table I)¹².

The diet offered to the FG had a concentration of 25% of flaxseed flour that aimed to meet the entire recommended fiber intake. Moreover, this amount of flaxseed flour lipid supplies the needs of animals and provides a diet with a high content of ALA and low LA, whereas the addition of soy oil in the control diet provides an inverse proportion.

Blood collection and sample processing

The rats were anesthetized with an intraperitoneal injection of Thiopentax® (Sodium Thiopental 1G, Cristália Chemicals Pharmaceuticals LTDA, Brazil) at 5% (0.15 ml/100 g pc, ip) for blood collection by cardiac

Table I
Control and flaxseed diets composition of each 100 g of diet

Nutrients	AJN-93 G		AJN 93-M	
	Casein (g)	Flaxseed (g)	Casein (g)	Flaxseed (g)
Casein ¹	20	14.11	11.8	5.9
Flaxseed ²	0	25	0	25
Starch ³	52.95	45.84	61.2	54.1
Refined Sugar ⁴	10	10	10	10
Minerals Mix AJN 93G ¹	3.50	3.50	3.50	3.50
Vitamins Mix ¹	1	1	1	1
Soy Oil ⁵	7	0	7	0
Cellulose ⁶	5	0	5	0
Choline Bitartrate ¹	0.25	0.25	0.25	0.25
Cystine ¹	0.30	0.30	0.18	0.18
<i>Tert</i> -Butyl hydroquinone	0.0014	0.0014	0.0008	0.0008
Total	100	100	100	100
Macronutrient Composition (100 g/diet)				
Protein	19.53	18.36	11.68	11.00
Fat	19.46	22.28	19.75	22.54
Carbohydrate	61.01	59.36	68.57	66.46
Total energy (kJ/100 g)	1488.83	1581.1	1485.9	1579.47

Ingredients used in diets preparation were provided by ¹M. Cassab Comércio e Indústria Ltda (São Paulo, SP, Brazil); ²Arma Zen Produtos Naturais Ltda. (Rio de Janeiro City, Rio de Janeiro State, Brazil); ³Maisena da Unilever Bestfoods Brazil Ltda (Mogi Guaçu City, São Paulo State, Brazil); ⁴União (Rio de Janeiro, RJ, Brazil); ⁵Liza da Cargill Agricultura Ltda. (Mairinque City, SP, Brazil); and ⁶Microcel da Blanver Ltda (Cotia City, SP, Brazil).

puncture and the blood was placed in tubes without anticoagulant. The collected blood was centrifuged (Sigma centrifuge) at 3500 rpm for 15 minutes to obtain serum, which was stored at -20°C. Analyses of cholesterol, triglycerides and high density lipoprotein (HDL) were performed with the use of the kits BIOCLIN® (Quibasa Industry - Basic Chemicals LTDA / Brazil). The concentration of very low density lipoprotein (VLDL) cholesterol was calculated according to Friedewald et al¹³. The glucose was measured by using the test strips glucometer brand Accu-Chek Active (Roche Diagnostics, Germany).

Visceral fat mass

Fat mass was removed and weighed when the animals were sacrificed. We considered all fats retroperitoneal, epididymal and mesenteric.

Histological Methods

The thoracic aorta fragments were fixed in buffered formaldehyde (formalin of Millonig) for 24 h, and processed according to standard histological technique

for paraffin as used by Pereira et al¹⁴. After inclusion, the paraffin blocks containing the fragments of aorta were cut in microtome CUT 4050 (Microtec®), in 5 μm sections and mounted on glass slides for optical microscopy.

The slides were stained by hematoxylin - eosin (overall), Weigert's resorcin fuchsin (elastic fiber staining). After, capturing images through a microscope Olympus BX40® coupled to a digital camera Lumenera®, using 20X objective. Histomorphometry scan was used to measure the thickness of the aorta through software Image J® (U.S. National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis

Data are reported as means and standard deviations. The results were tested for normal distribution using the Shapiro-Wilk test. For results with normal distribution, comparisons between groups were carried out using Student's *t* test for independent data. Where results did not have normal distribution, the Mann-Whitney nonparametric test was employed. In all tests, significance was set at $p \leq 0.05$. SPSS for Windows, version 10.0, was used for statistical analysis.

Table II
Effect of diets on weight and biochemical parameters of the animals at the end of the experiment

	FG (n = 10)	CG (n = 10)	P
Final body weight (g)	522.4 ± 54.3	654.2 ± 77.2	p < 0.000
Cholesterol (mg/dL)	68.9 ± 10.6	108.5 ± 21.5	p < 0.000
Triglyceride (mg/dL)	46.4 ± 8.9	66.2 ± 8.5	p < 0.000
HDL (mg/dL)	57.9 ± 11.7	84.3 ± 37.3	p = 0.046
VLDL (mg/dL)	8.4 ± 3.3	12.1 ± 4.2	p = 0.028
Glucose (mg/dL)	95.7 ± 5.3	103.2 ± 6.6	p = 0.004

Data are presented as mean ± standard deviation of 10 rats per group.
CG: Control group; FG: Flaxseed group.

Results

At 250 days of life, FG had 20% less body weight when compared to the CG (table II). The FG had lower values in visceral fat mass (- 44%, FG = 40.3 ± 10.9 g, CG = 71.9 ± 22.2 g, p = 0.0016) and these were maintained even after the calculation of relative fat mass (-29%, FG = 7.6 ± 1.7 g/100 g body weight, CG = 10.8 ± 2.3 g/100 g body weight, p = 0.0016).

Table II shows that the flaxseed group showed changes in biochemistry parameters. As regards the lipid profile of the FG showed changes in all parameters. The FG lower values for cholesterol (p < 0.000), triglycerides (p < 0.000), HDL (p = 0.046) and VLDL (p = 0.028). The concentration of serum glucose was lower in the FG (p = 0.004).

At 250 days it was observed in the flaxseed group a smaller thickness of the aorta (GL = 0.13 ± 0.01 mm; GC = 0.15 ± 0.02 mm; p < 0.005) (fig. 1).

Discussion

Supplementation with flaxseed flour or food source of ALA in the cardiovascular system has been investigated by many researchers, but studies only evaluate this short-term consumption. The differential of this research was the study of the effects of this prolonged consumption in healthy mice as a preventive measure in parameters related to cardiovascular risk. The flaxseed flour shown to reduce body weight gain and

visceral fat accumulation, improve lipid profiles, lower blood glucose and in protect against the thickening of artery.

The reduction in body weight can be attributed to the fact that the flaxseed contains 28% fibers, and these can promote the control of energy intake, increase satiety and reduce the risk of developing obesity. These functions may occur due to the physicochemical properties of the fibers by forming a gel indicate a feeling of early satiety and prolonged^{15,16}.

In the present study it was observed lower accumulation of visceral fat. Few studies describe the possible protective effect of this oilseed on adiposity. The administration of secoisolariciresinol diglycoside (SDG), which is the main lignan found in flaxseed reduced the accumulation of visceral fat mass in mice and rats^{10,17}. No statistical differences were found in visceral fat mass of rats that received the seed components only during lactation, although there was, in FG, a numerical reduction of 21.43%¹⁸. The same tendency was shown in mice that received a diet containing flaxseed oil compared to mice fed a diet rich in saturated fats¹⁹.

It has been suggested that flaxseed and its components can reduce the risk of cardiovascular diseases²⁰, but the mechanisms of this reduction are not yet well established. Some authors attribute the decrease in risk with improved lipid profile⁸. The effect of flaxseed on the lipid profile has been investigated extensively and assigned not only to seed flour, but also to SDG, its oil and its protein. It has been found in the present study a 36% reduction in total cholesterol, 29% in triglycerides

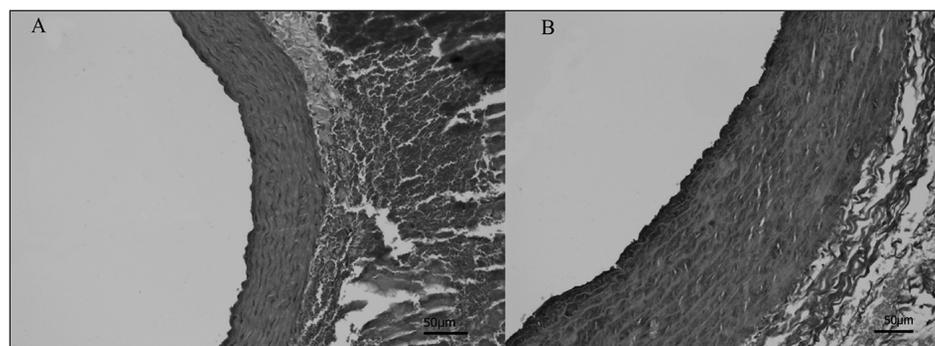


Fig. 1.—Photomicrography of the rat's aorta. The flaxseed group (A) showed a smaller thickness compared to the control group (B).

and 30% in VLDL in the FG when compared to the values of the group that not consumed the seed.

The use of 20 g of flaxseed for two months in hyperlipidemic patients resulted in modification of cardiovascular risk factors, with significant reduction of cholesterol, low density lipoprotein (LDL) and triglycerides²¹.

Flaxseed oil reduced total cholesterol and low density lipoprotein in mice that consumed a diet rich in fat and protect against renal lesions associated with hypercholesterolemia²².

Despite the improvement in lipid profile, the FG had lower HDL in the present study. A survey conducted by Bloedon et al²³. showed negative effect of flaxseed consumption on the concentration of HDL, when flaxseed was supplied at a dose of 40 g / day in hypercholesterolemic humans. A similar result was found when women consumed 25 g of defatted flaxseed for 12 weeks²⁴. There was no improvement in HDL after use of the linseed oil associated with a diet high in fat²². A contrary result was found in hamsters that fed 15% flaxseed for twenty weeks and showed 91% increase in HDL compared to the control group²⁵.

In this study, adult rats fed flaxseed showed, when compared to the control group, a 7% reduction in blood glucose. These results suggest a beneficial effect of flaxseed flour consumption on the regulation and maintenance of glycemic homeostasis. As well as Abuelgassim²⁶ who also noted that the flaxseed extract caused a reduction in glucose concentrations of diabetic and nondiabetic mice.

Aging is generally associated with changes in the cardiovascular system, particularly in the structure and function of the arteries. Histological analysis revealed a smaller thickness of the aorta in rats fed with a diet rich in flaxseed. This result suggests that a diet with 25% of this oilseed has cardioprotective effect against this thickening of artery.

In another study, the animals that consumed a diet rich in phytoestrogens for a prolonged period had a lower thickness of the aorta when compared to the control group²⁷. Faintuch et al²⁸. noted that obese patients who consumed the golden flaxseed for 12 weeks showed no changes in carotid diameter. Prim et al²⁹. demonstrated that flaxseed supplementation in rabbits fed with hypercholesterolemic diet for one month did not alter the thickness of the intima and the ratio of the area of aorta intima-media.

Winnik et al³⁰., using a rat model of developing atherosclerosis, demonstrated that supplementation with ALA decreased the formation of atheroma plaques.

A limiting factor to be considered in this study is that the imbalance observed between u-6 and u-3 cannot be extrapolated to humans, because flaxseed was the only lipid source in the diet. Human food has a greater variety of lipid sources. Additionally, the highest percentage of u-6 soybean oil may modulate to a greater inflammatory response in the control group. However, the u-6/u-3 ratio must be observed by respecting the needs of the organism.

Conclusions

So the extrapolation of results from animal studies to humans must be made with caution, but this study in healthy rats showed that the flaxseed flour consumed for a prolonged period decreased the thickness of the aorta and may be used as a preventive measure in modulating some modifiable risk factors related to cardiovascular disease.

Acknowledgments

Research was supported by the Coordination for the Personnel Improvement of Higher Education (CAPES), the State of Rio de Janeiro Carlos Chagas Filho Research Foundation (FAPERJ), and the National Council for Scientific and Technological Development (CNPq).

References

1. World Health Organization. Enfermedades cardiovasculares. 2011, <http://www.who.int/mediacentre/factsheets/fs317/es/index.html>.
2. Grech ED. Pathophysiology and investigation of coronary artery disease. *BMJ* 2003; 326 (7397): 1027-30.
3. Tsamis A, Rachev A, Stergiopoulos N. A constituent-based model of age-related changes in conduit arteries. *Am J Physiol Heart Circ Physiol* 2011; 301: H1286-H1301.
4. Strait JB, Lakatta EG. Aging-associated cardiovascular changes and their relationship to heart failure. *Heart Fail Clin* 2012; 8: 143-64.
5. Rankin P, Morton DP, Diehl H et al. Effectiveness of a volunteer-delivered lifestyle modification program for reducing cardiovascular disease risk factors. *Am J Cardiol* 2012; 109 (1): 82-6.
6. Carter JF. Potential of flaxseed and flaxseed oil in baked goods and other products in human nutrition. *Cereal Food World* 1993; 38 (10): 753-9.
7. Hutchins AM, Brown BD, Cunnane SC et al. Daily flaxseed consumption improves glycemic control in obese men and women with pre-diabetes: a randomized study. *Nutr Res* 2013; 33: 367-75.
8. Cardozo LFMF, Chagas MA, Soares LL et al. Exposure to flaxseed during lactation does not alter prostate area or epithelium height but change lipid profile in rats. *Nutr Hosp* 2010a; 25 (2): 250-5.
9. Taylor CG, Noto AD, Stringer DM et al. Dietary milled flaxseed and flaxseed oil improve n-3 fatty acid status and do not affect glycemic control in individuals with well-controlled type 2 diabetes. *J Am Coll Nutr* 2012; 29 (1): 72-80.
10. Fukumitsu S, Aida K, Ueno N et al. Flaxseed lignan attenuates high-fat diet-induced fat accumulation and induces adiponectin expression in mice. *Br J Nutr* 2008; 100: 669-76.
11. National Research Council (US) Institute for Laboratory Animal Research. Guidance for the Description of Animal Research in Scientific Publications. Washington (DC): National Academies Press (US). 2011, <http://www.ncbi.nlm.nih.gov/books/NBK84205/>.
12. Reeves PG, Nielsen FH, Fahey Jr GCF. AIN-93 purified diet of laboratory rodents: final report of the American Institute of Nutrition ad hoc Writing Committee on the Reformulation of the AIN-76A rodents diet. *J Nutr* 1993; 123 (11): 1939-51.
13. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of lowdensity lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.

14. Pereira LM, Viana GM, Mandarim-de-Lacerda CA. Morphology and stereology of the myocardium in hypertensive rats. Correlation with the time of nitric oxide synthesis inhibition. *Arq Bras Cardiol* 1998; 70: 397-402.
15. Kristensen M, Jensen MG. Dietary fibres in the regulation of appetite and food intake. Importance of viscosity. *Appetite* 2011; 56: 65-70.
16. Ibrügger S, Kristensen M, Mikkelsen MS et al. Flaxseed dietary fiber supplements for suppression of appetite and food intake. *Appetite* 2012; 58: 490-5.
17. Park JB, Velasquez MT. Potential effects of lignan-enriched flaxseed powder on bodyweight, visceral fat, lipid profile, and blood pressure in rats. *Fitoterapia* 2012; 83: 941-6.
18. Cardozo LFMF, Soares LL, Chagas MA et al. Maternal consumption of flaxseed during lactation affects weight and hemoglobin level of offspring in rats. *J Pediatr* 2010b; 86 (2): 126-30.
19. Tzang BS, Yang S F, Fu SG et al. Effects of dietary flaxseed oil on cholesterol metabolism of hamsters. *Food Chem* 2009; 114: 1450-5.
20. Patade A, Devareddy L, Lucas EA et al. Flaxseed reduces total and LDL cholesterol concentrations in native american postmenopausal women. *J Womens Health (Larchmt)* 2008; 17 (3): 355-66.
21. Mandasescu S, Mocanu V, Dascalita AM et al. Flaxseed supplementation in hyperlipidemic patients. *Rev Med Chir Soc Med Nat Iasi* 2005; 109 (3): 502-6.
22. Akpolat M, Kanter M, Topcu-Tarladacalisir Y et al. Protective effect of flaxseed oil on renal injury in hyperlipidaemic rats: The effect of flaxseed oil on hyperlipidaemia. *Phytother Res* 2011; 25 (6): 796-802.
23. Bloedon LT, Balikai S, Chittams J et al. Flaxseed and cardiovascular risk factors: Results from a double blind, randomized, controlled clinical trial. *J Am Coll Nutr* 2008; 279 (1): 65-74.
24. Simbalista RL, Sauerbronn AV, Aldrighi JM et al. Consumption of a flaxseed-rich food is not more effective than a placebo in alleviating the climacteric symptoms of postmenopausal women. *J Nutr* 2010; 140: 293-7.
25. Haliga R, Mocanu V, Oboroceanu T et al. The effects of dietary flaxseed supplementation on lipid metabolism in streptozotocin-induced diabetic hamsters. *Rev Med Chir Soc Med Nat Iasi* 2007; 111 (2): 472-6.
26. Abuelgassim AO. Effect of flax seeds and date palm leaves extracts on serum concentrations of glucose and lipids in alloxan diabetic rats. *Pakistan J Biol Sci* 2010; 13 (23): 1141-5.
27. Daleprane JB, Chagas MA, Vellarde GC et al. The impact of non- and genetically modified soybean diets in aorta wall remodeling. *J Food Sci* 2010; 75 (7): T126-T131.
28. Faintuch J, Bortolotto LA, Marques PC et al. Systemic inflammation and carotid diameter in obese patients: pilot comparative study with flaxseed powder and cassava powder. *Nutr Hosp* 2011; 26 (1): 208-13.
29. Prim CR, Baroncini LA, Précoma LB et al. Effects of linseed consumption for a short period of time on lipid profile and atherosclerotic lesions in rabbits fed a hypercholesterolaemic diet. *Br J Nutr* 2012; 107: 660-4.
30. Winnik S, Lohmann C, Ricther EK et al. Dietary α -linolenic acid diminishes experimental atherogenesis and restricts T cell-driven inflammation. *Eur Heart J* 2011; 32 (20): 2573-84.