Sys Rev Pharm 2021;12(3):196-206 A multifaceted review journal in the field of pharmacy

A Vital Role Of Secoisolariciresinol Diglucoside Against Cardiotoxicity

Zahraa Ahmed Okhti¹ and Yassir M K Al-Mulla Hummadi²

¹ College of Pharmacy, Department of Clinical Laboratory Sciences, Mustansiriyah University, Iraq ² College of Pharmacy, Department of pharmacology and Toxicology, Mustansiriyah University, Baghdad- Iraq. Corresponding author: Zehraa.ahmed @uomustansiriyah.edu.iq,

ABSTRACT

Cardiovascular dysfunction is one of the most risk that has been faced human in recent time, however new drugs discovery and medicine development ,but still need to natural compound to avoid or moderate deterioration of cardiovascular ,for that researches has been set out. to display effective role of secoisolariciresinol diglucoside(SDG)against hypercholesterolemia and inflammatory cell infiltration, this work has been done. Treatment role of SDG is designed by assayed lipid profile and histopathological diagnosis of heart tissue in animals treated with high cholesterol diet (HCD).

Animal experimental

Twenty male rabbits were divided into four group, (5 rabbit for each group) normal diet for 1 month, control administrated certain diet: negative control: 1600 mg of cholesterol with normal diet for 1 month. Group III - a positive normal diet with 1600 mg of cholesterol for 1 month then received orally with 40mg/kg/day of partial pure SDG for two weeks. Results:

lipid profile(TC,TG,HDL,LDL) content has been found significantly increased to(1130.5, 135.3, 201.2 and 901.74) mg/dl respectively, after HCD (control II).on another side,has been noticed post-administrated partial pure SDG ,decreased levels of (TC,TG,HDL,LDL) to(57,44, 43, and 4.5) mg/dl respectively,these parameters indicated that partial pure SDG able to decrease the level of all lipid profile parameters. in addition ,observed hemorrhage coagulative necrosis with severe fatty change and inflammatory cells infiltration in heart after HCD, While animals deals with partial pure SDG did not shows any histology alteration . Conclusion:

From our research, can conclude that partial pure SDG has been maintain lipid profile parameters normally and decreased signs of inflammation in various site of heart.

INTRODUCTION

Recent today, Cardiovascular diseases have become a growing problem and regarded major cause of morbidity and mortality mainly in varies countries (1;2). Main risk leading to development of cardiovascular diseases is Hypercholesterolemia (3,4). Oxidative stress induced by reactive oxygen species as well as, plays a vital role in the etiology of atherosclerosis and heart lesion (5). Myocardium injury may have effected by Total cholesterol and other lipids (6). saturated fatty acids diet leading to Reducing risk of cardiovascular disease.(7) fatty change induced in different organs such as the liver, heart, and kidney by Hyperlipidemia[8,9].Previous Experimental hypercholesterolemia are correlation with elevated of production of free radical and decreased activities of endogenous radical scavengers (10). Elevated LDL value may be represented the harmful agent for atherosclerosis and endothelial dysfunction (11).To improve level of total cholesterol, recommended changes lifestyle such as reduce smoking rate, reducing alcohol consumption, increased physical exercise and health diet (12).However, most people could not management and control their blood cholesterol. For that, tended to use medical plant because, they have antioxidant compounds and hypolipidemic effect. Among medical plant, Linum usitatissimum L.which are contain fiber and oil, and food (13).

Linum usitatissimum L. contain different active compounds and elements including cyanogenic glycosides, linolenic acid ,alkaloids, secoisolariciresinol **Keywords:** High cholesterol diet ,Lipid profile , SDG, heart fatty change.

Correspondence:

Zahraa Ahmed Okhti

College of Pharmacy, Department of Clinical Laboratory Sciences, Mustansiriyah University, Iraq

*Corresponding author: Zahraa Ahmed Okhti email-address: Zehraa.ahmed @uomustansiriyah.edu.iq

diglucoside (SDG),polysaccharides, cyclic peptides. Previous studies of Linum usitatissimum L. have focused on extract products containing SDG or a-linolenic acid. To our best knowledge, there is no document about use SDG as anti-hyperlipidemic agent for fatty heart report in Iraq. Therefore, the present study direct to clear treatment exert of SDG on high cholesterol that trigger heart tissues damage through histopathological study.

MATERIAL AND METHOD

Extraction of secoisolariciresinol diglucoside(SDG)

extracted SDG by taken (50) g amount of defatted flaxseed, processing with aqueous methanol, stirring for four hours and centrifugation at $10,000 \times g$ for 5 min, (20 mM) of sodium hydroxide were added at 50 ° C. (Li *et al.*,2008,14).,finally dissolve 0.04g of SDG in D.W. to obtain (40mg/ml).(Prasad,2009,15).

Experimental protocol

experimental design on (20) male Pathogen Free rabbits were divided into four groups {(5) rabbits for all group}which administrated one of following diet: Group I-a normal diet free cholesterol for 1 month (negative control),Group II- a normal diet with 1600 mg/kg cholesterol for 1 month.(control positive),Group III - a normal diet contain with 1600 mg/kg cholesterol for 1 month then received orally with 40mg/kg/day of SDG for two weeks.

Lipid profiles assay

Serum was estimated to determined total cholesterol, triglyceride ,high density lipoprotein by using Biommaghreb kits [15]. Serum LDL-C was determined by next calculation:

"LDL – C (mg/dl) = TC – (HDL – C + Triglycerides)

5 Histopathological Examination

Heart removed after heart puncture ,complete fixation step and sectioning at 4-6 μ m thickness then stained with routing stained and examined under light microscope (Carleton *et al.*, 1980,19).

Statistical analysis

All the tests were three replicates estimations and the outcomes have appeared as the mean \pm S.E. Data was analysis with (one way ANOVA) and SPSS statistical program. A difference considered is a significance at P<0.05.

RESULTS

lipid profile

From our results which shown in Figure(1,2,3,4) were found lipid profile that include (total cholesterol (TC), triglyceride(TG), HDL and LDL) level in negative control was (55, 44, 43.8 and 5.4) mg/dl respectively, while their levels were significantly elevated ($P \le 0.05$) to (1130.5, 135.3, 201.2and 830.5) mg/dl respectively after treatment with high cholesterol. In another side, post-administrated SDG (total cholesterol, triglyceride(TG), HDL and LDL) level were significantly decreased ($P \le 0.05$) to (50, 46.8, 37.5 and 7.47) mg/dl respectively.



Fig.(1): demonstrated level of (TC) before and post treatment with SDG. Time represent: 0 days: (1) normal feed) ;(2) 4 weeks :1600mg/ml cholesterol diet for all groups except groupI(Control negative); (3) 6weeks :treatment with partial pure SDG for 14 days after 4 weeks of cholesterol diet according to groups treatment program.



Fig(2) : demonstrated level of (TG) before and post treatment with SDG . Time represent: 0 days :normal feed (negative Control); 4 weeks :1600mg/ml cholesterol diet for all groups except group I(Control negative);

6weeks :treatment with partial pure SDG $\,$ for 14 days after 30days of cholesterol diet according to groups treatment program. .



Fig.(3): demonstrated level of (HDL)before and post treatment with SDG. Time represent: Time represent: 0 days: (1) normal feed) ;(2) 4 weeks :1600mg/ml cholesterol diet for all groups except groupI(Control negative); (3) 6weeks :treatment with partial pure SDG for 14 days after 4 weeks of cholesterol diet according to groups treatment program.



Fig.(4): demonstrated level of (LDL)before and post treatment with SDG. Time represent: 0 days: (1) normal feed) ;(2) 4 weeks :1600mg/ml cholesterol diet for all groups except groupI(Control negative); (3) 6weeks :treatment with partial pure SDG for 14 days after 4 weeks of cholesterol diet according to groups treatment program.

Histopathological Sections

Histopathological section in heart showed hemorrhage between cardiac muscle fibers with coagulative necrosis some of cardiac fibers(Figure:6,13) with severe fatty infiltration in atrium (Figure:8),The aorta of animal adminstration with cholesterol revealed RBCs ,inflammatory cells and vacoules of fat droplets in subintima(Figure:8) with fibrosis in subintima (Figure:9).in other section, RBCs and inflammatory cells in the pericardium of atrium were seen (Figure: 10),in addition to severe congestion of blood vessels with vacuole of fat droplets in their lumen(Figure:11),Also ,it was marked fatty changes in cardiac muscle fibers (Figure:14). While , in heart of animal adminstration with cholesterol and treatment with SDG partial pure shows no any histology alteration , the muscle fibers are normal and normal nuclei . (H&E stain 40X).In addition ,histopathological section in heart of animal adminstration with cholesterol and treatment partial pure of SDG,expressed fatty changes in cardiac fibers(Figure:16) with mononuclear cells infiltration in the pericardium(Figure:17 and normal intima of congested blood vessels (Figure:18)



Fig.(5): section in heart of control negative animal shows no clear lesions (H&E stain 40X).



Fig.(6):.Histopathological section in heart of animal adminstration with cholesterol shows hemorrhage between cardiac muscle(blue arrow) with coagulative necrosis some cardiac fibers (pink arrow) (H&E stain 40X)

A Vital Role Of Secoisolariciresinol Diglucoside Against Cardiotoxicity



Fig. (7):.section in aorta of animal adminstration with cholesterol shows RBCs ,inflammatory cells and vacoules of fat droplets in subintima (blue arrow) (H&E stain 40X).



Fig(8): section in heart of animal adminstration with cholesterol shows severe faty infiltration in atrium (blue arrow) (H&E stain 40X).



Fig.(9): section in aorta of animal adminstration with cholesterol shows large , vacoules of fat droplets(blue arrow) and fibrosis (pink arrow) in subintima (H&E stain 40X).

A Vital Role Of Secoisolariciresinol Diglucoside Against Cardiotoxicity



Figure(10): section in heart of animal adminstration with cholesterol shows RBCs and inflammatory cells in the pericardium of atrium(blue arrow), (H&E stain 40X)



Fig. (11): section in heart of animal adminstration with cholesterol shows severe congestion of blood vessels with vacuole of fat droplets in their lumen(blue arrow)(H&E stain 40X)



Fig.(12):. section in heart of animal adminstration with cholesterol shows marked fatty changes in cardiac muscle fibers (blue arrow) (H&E stain 40X)



Fig.(13): section in heart of animal adminstration with cholesterol shows marked fatty changes in cardiac muscle fibers (blue arrow) (H&E stain 40X)



Fig.(14):section in heart of animal adminstration with cholesterol shows hemorrhage between cardiac muscle(blue arrow) with necrosis of some cardiac fibers(pink arrow)(H&E stain 40X).



A Vital Role Of Secoisolariciresinol Diglucoside Against Cardiotoxicity



Fig.(15):section in heart of animal adminstration with cholesterol and treatment with partial pure SDG shows no clear lesions, the muscle fibers are normal and normal nuclei (H&E stain 40X).



Fig.(16):section in heart of animal adminstration with cholesterol and treatment with SDG partial pure shows fatty changes in cardiac fibers(blue arrow). (H&E stain 40X)



Fig. (17):. section in heart of animal adminstration with cholesterol and treatment with SDG partial pure shows mononuclear cells(blue arrow) infiltration in the pericardium (H&E stain 40X)



Fig. (18): section in heart of animal adminstration with cholesterol and treatment with partial pure SDG shows normal intima of congested blood vessels (blue arrow)(H&E stain 40X).

DISCUSSION

fasting food containing high cholesterol that contributes to development cardiovascular disease which is currently being reassessed in medical circles ,high cholestrol diet leading to Serum cholesterol rising , that has been known to give atherosclerosis and induce oxidative stress which are known to have an harmful effects on the architecture of cells(20).serums of experimental animals are contain high level of cholesterol, triglyceride and LDL-cholesterol that have been observed in Cholesterol feeding rabbits as well as, These results that consistent with others reports (21,22). Also, organs function becomes impaired when gradually diminished blood supply to the organs.(23-26). furthermore, a high cholesterol(HC) diet lead to cell death and steatosis (27). Our findings agreement with previous reports that indicated cholesterol accumulation in the aorta of rabbits fed an HC. Male rabbits are most sensitive to show hypercholesterolemia through few days and to the evolution of atherosclerosis after high cholesterol feeding (20) that mimics alteration occur in human than more rat and mice (28 and 29), for these reasons chose these animals as model for hypercholesterolemia and pathological change in heart. For instance, rabbits are prone to exhibit hypercholesterolemia because high aggregation of practices of exogenous cholesterol and insufficient amount of sterols.(30). Also, our reaserch results were similar to others findings of previous studies that have been showed cholesterol fed rabbits induced fatty change in liver (31) and heart injury.beside that ,libby ,2002 also cleared that inflammation may be connected between atherosclerosis and hypercholestrolemia(32).Morever, triglyceride and low density are stimulated leukocytes adhesion to surface of endothelial(33)as well as, started earlier in the myocardium and including deterioration of muscle cells,coagulative necrosis of cardiomyocytes and infiltration of macrophages 34).In (addition,Aydan,2004(35)showed that increase inflammatory cells and vacoules of fat droplets and fibrosis of subintima in aorta of hypercholestermia rabbits models . furthermore , Gamal , 2014 (36) reported that Severe fatty infiltration in the atrium, RBCs and inflammatory cells in the pericardium of atrium was also shown after high cholesterol administration, these inflammatory processes caused by high cholestrol diet that leading to bind and to penetrat leukocytes to layer of endothelium in early atherosclerotic and detected monocytes attached to endothelial cells(37) ,monocytes moves to subendothelial space and differentiated to macrophages, these cell can be loaden fat droplit .further more , showed that leukocytes are appeared near to aortas after three weeks of 0.2 % cholestrol intake and foam cell and monocytes in subedothelial space (38). On other hand, lipid level can be reduced by provision antioxidant, supplementation with L- arginine and nitric oxide.cholesterol ester and cholesterol can be reduced in liver by inake SDG(39).Prasad ,2005(40) elucidated that flax lignan reduce cholesterol level of rabbits intake 40 mg/kg daily orally for two month ,in addition SDG modulated hypercholesterolemia and decrease progression of arteriosclerosis in experimental rabbits daily orally (40) Moreover, received 40 mg/kg Roseingnolie et al.,(1987) finding that polyphenol modulated inflammatory mediators and monocytes infiltration.(41).SDG decrease cholesterol accumulation in the aortas and oxidative stress of rabbits fed high cholesterol diet. Lucas et al., (2004) (42) showed that SDG have benefit role to reduced risk of cardiovascular disease (CVD)through its free radical scavenger activity ,cholesterol lowering or may be by suppression peroxidation and reducing oxidized LDL(43)other works clarify SDG exert by reduce of monocytes adhesion to the endothelium (44). Besides that, atherosclerotic lesions and plaques may reduce by SDG

CONCLUSION

The results showed a cytoprotective action of the partial pure SDG, may be due to free radical elimination and anti-inflammatory properties of SDG.

ACKNOWLEDGMENT

The authors are thankful towards Almustansiriyah Unive rsity for facilitating and presenting a practical forum to finish this research.

REFERENCES

- O'Donnell, C.J. and E.G. Nabel., 2011. Genomics of cardiovascular disease. N. Engl. J. Med., 365: 2098-2109.
- Nabel, E.G. and E. Braunwald., 2012. A tale of coronary artery disease and myocardial infarction. N. Engl. J. Med., 366: 54-63.

- 3. Freedman, J.E. 2003. High-fat diets and cardiovascular disease. Journal of the American College of Cardiolog., 41(10): 1750-1752.
- 4. Vijayakumar, R.S. ; Surya, D. and Nalini, N. 2004 . Antioxidant efficacy of black pepper (*Piper nigrum L.*) and piperine in rats with high fat diet induced oxidative stress. Redox Report ., 9 (2) : 105–110.
- Du, H. ; Zhao, X. ; You, J.S. ; Park, J.Y. ; Kim, S.H. ; and Chang, K.J.,2010. Antioxidant and hepatic protective effects of lotus root hot water extract with taurine supplementation in rats fed a high fat diet. Journal of Biomedical Science., 17, supplement 1, p: S39.
- Toda, E., T. Toru, R.B. Singh, S.E. Alam and F.D. Meester *et al.*, 2012. Paleolithic-style diet and coronary artery disease: The tissue is the issue? Am. Med. J., 3: 183-193. DOI:10.3844/amjsp.2012.183.193.
- Cheema, S.K., 2012. Dietary fats and heart disease: From prenatal to postnatal environment. Am.Med. J., 3: 175-182. DOI: 10.3844/amjsp.2012.175.182
- 8. Badimon, L. ; Vilahur, G. and Padro, T. 2010. Nutraceuticals and atherosclerosis: human trials.Cardiovascular Therapeutics., 28 (4): 202-215.
- 9. Kwiterovich, P.O. and Jr, P.O. 1997. The effect of dietary fat, antioxidants, and pro-oxidants on blood lipids, lipoproteins, and atherosclerosis. Journal of the American Dietetic Association ., 97 (7): S31–S41.
- 10. Napol, C., J.L. Witztum, F. De Nigris, G. Palumbo and F.P. D'Armiento *et al.*, 1999. Intracranial arteries of human fetuses are more resistant to hypercholesterolemia-induced fatty streak formation than extracranial arteries. Circulation, 99: 2003-2010. DOI: 10.1161/01.CIR.99.15.2003
- Bonetti, P.O., L.O Lerman and A. Lerman, 2003. Endothelial dysfunction: A marker of atherosclerotic risk. Arteriosclerosis. Thromb. Vascular Biol., 23: 168-175.

DOI:10.1161/01.ATV.0000051384.43104.FC

- Kabiri, N. ; Asgary, S. and Setork, M.,2011. Lipid lower ng by hydroalcoholic extracts of *Amaranthus Caudatus L.* induces regression of rabbits atherosclerotic lesions. Lipids in Health and Disease , 10:89.
- 13. Oomah, B. D., 2001. Flaxseed as a functional food source. Journal of the Science of Food and Agriculture, 81, 889e894.
- 14. Li X, Yuan J.P., Xu,S.P., Wang,J.H.and Liu X.,2008. Separation and determination of secoisolariciresinol diglucoside oligomers and their hydrolysates in the flaxseed extract by high-performance liquid chromatography. *Journal of Chromatography* A, 1185:223–232.
- 15. Prasad, K.,1999. Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. *Circulation* 99, 1355-1362.
- 16. Flegg, H.M.,1973.An investigation for the determination of serum cholesterol by an enzyme method.*Ann Clin Biochem.*; 10:79-84.
- Friedewald WT; Levy R.I.; Fredrickson D.S..1972.Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* Jun; 18(6):499-502
- Fossati, P. and Prencipe, L.,1982. Serum triglycerides determined colorimetrically with an enzyme that produceshydrogen peroxide. Cli Chem., 28:2077-88.

- Carleton, H.M., R.A. Drury and E.A. Wallington, 1980. Carleton's Histological Technique. 5th Edn., Oxford University Press, ISBN-10: 0192613103, pp: 520
- Aikawa, M., Fukumoto, Y., Rabkin, E., and Libby, P.,2000. Rabbit models of atherosclerosis. In "Contemporary Cardiology: Vascular Disease and Injury, Preclinical Research", eds. Simon, D. I., and Rogers, S. C., Humana Press Inc., Totowa, New Jersey, pp. 175–191
- 21. Vijaimohan K., Jainu M., Sabitha K. E., Subramaniyam S., Anandhan C. and Shyamala Devi C. S.,2006. Beneficial effects of alpha linolenic acid rich flax seed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rat. *Life Sci.* 79, 448-454.
- 22. Jain G.C., Jhalani S., Agarwal S.and Jain K., 2007. Hypolipidemic and Antiatherosclerotic Effect of *Leptadenia pyrotechnica* Extract in Cholesterol Fed Rabbits. *Asian J. Exp. Sci.*, 21, 1: 115-122.
- 23. Park, T. and K. Lee, 1998. Dietary taurine supplementation reduces plasma and liver cholesterol and triglyceride levels in rats fed a highcholesterol or a cholesterol-free diet. Adv. Exp.Med. Biol., 3: 319-325. PMID: 9635047
- 24. Montilla, P., I. Espejo, M. C. Munoz, I. Bujalance and J.R. Munoz-Castaneda *et al.*, 2006. Protective effect of red wine on oxidative stress and antioxidant enzyme activities in the brain and kidney induced by feeding high cholesterol in rats. Clin. Nutr., 25: 146-153.
- 25. Prasad, K.,2008. Regression of hypercholesterolemic atherosclerosis in rabbits by secoisolaricresinol diglucoside isolated from flaxseed. Atherosclerosis, 197: 34-42.
- Stapleton, P.A., A.G. Good Will, M.E. James, R.W. Brock and J.C. Frisbee., 2010. Hypercholesterolemia and microvascular dysfunction: Interventional strategies. J. Inflam., 7: 54-54.
- Mei, S., H.M. Ni, S. Manley, A. Bockus and K.M. Kassel et al., 2011. Differential roles of unsaturated and saturated fatty acids on autophagy and apoptosis in hepatocytes. J. Pharmacol. Exp. Ther., 339: 487-498.
- Bocan TMA, Mazur MJ, Mueller SB, Brown EQ, Sliskovic DR, O'Brien PM, Creswell MW, Lee H, Uhlendorf PD, Roth BD, Newton RS., 1994 .Antiatherosclerotic activity of inhibitors of 3-hydroxy-3methylglutaryl coenzyme A reductase in cholesterolfed rabbits: a biochemical and morphological evaluation. *Atherosclerosis* 111, 127–42
- Yanni AE., 2004 .The laboratory rabbit: an animal model of atherosclerosis research Lab Anim. 38(3):246-56
- Kolodgie FD, Katocs AS, Largis EE, Wrenn SM, Cornhill JF, Herderick EE, Lee SJ, Virmani R .,1996. Hypercholesterolemia in the rabbit induced by feeding graded amounts of low level cholesterol. *Arteriosclerosis Thrombosis and Vascular Biology* 16, 1454–64
- 31. Okhti ZA, Muthanna I, Al-Ezzi, Abdulmahdi R.,2016. The protective role of flaxseed Lignan in male rabbits with high-fat diet: Histopathological study. Int J Pharm Pharm Sci;8:90-4.
- 32. Libby P, Aikawa M .2002. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. *Nature Medicine* 8, 1257–62
- 33. Dart A. M. and Chin-Dusting J. P.F., 1999. Lipids and the endothelium. Cardiovascular Research 43 ,308– 322.

- Caligiuri, G., B. Levy, J. Pernow, P. Thoren and G.K. Hansson., 1999. Myocardial infarction mediated by endothelia receptor signaling in hypercholesterolemic mice. Proc. Nat. Acad. Sci. USA., 96: 6920-6924.
- 35. Hatipoglu, A., Ö. Kanbagli, J. Balkan, M. Küçük, U. Çevikbas, G. Aykaç-toker, H. Berkkan, M. Uysal.,2004. Hazelnut oil administration reduces aortic cholesterol accumulation and lipid peroxides in the plasma, liver, and aorta of rabbits fed a highcholesterol diet. *Biosci. Biotechnol. Biochem.* 68(10): 2050-2057
- 36. Gamal Hasan Abdel-Rahman.,2014. Taurine Attenuates Hepatic And Cardiac Damage And Apoptosis In Rabbits Fed A High-Fat Diet. Online Journal Of Biological Sciences 14 (1): 12-20,
- Scalia, R., Appel III, J. Z., & Lefer, A. M., 1998. Leukocyte-endothelium interaction during the early stages of hypercholesterolemia in the rabbit: role of P-selectin, ICAM-1, and VCAM-1. *Arteriosclerosis, thrombosis, and vascular biology, 18*(7), 1093-1100.
- Rosenfeld ME, Tsukada T, Gown AM, Ross R .,1987.,Fatty streak initiation in Watanabe heritable hyperlipidemic and comparably hypercholesterolemic fat-fed rabbits. *Arteriosclerosis* 7, 9–23
- Prasad, K., 2008. Regression of hypercholesterolemic atherosclerosis in rabbits by secoisolaricresinol diglucoside isolated from flaxseed. Atherosclerosis, 197: 34-42.
- Prasad K.,2005 Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. Atherosclerosis 179: 269-275.
- Rosignoli, P.; Fuccelli, R.; Fabiani, R.; Servili, M.; Morozzi, G., 2013. Effect of olive oil phenols on the production of inflammatory mediators in freshly isolated human monocytes. J. Nutr. Biochem., 24, 1513–1519.
- 42. Lucas, E.A., Lightfoot, S.A., Hammond, L.J., Devareddy, L., Khalil, D.A., Daggy, B.P., Smith B.J., Westcott, N., Mocanu, V., Soung, D.Y. and Arjmandi, B.H., 2004. Flaxseed reduces plasma cholesterol and atherosclerotic lesion formation in ovariectomized Golden Syrian hamsters. *Atherosclerosis* ,173, 223-229.
- 43. Teker, M. E., İnan, B., & İnan, Ö., 2017. Investigation of the impact of flaxseed (Linum usitatissimum) on the lower extremity ischemia/reperfusion injury in a hypercholesterolemic rat model. Journal of Vascular Medicine and Surgery, 5(5), 338.
- 44. Tangney, C.C.; Rasmussen, H.E., 2013.Polyphenols, inflammation, and cardiovascular disease. Curr. Atheroscler. Rep., 15, 324–340.