Iraqi National Journal of Chemistry

Journal homepage:
http://iqnjc.com/Default.aspx

Evaluation of Vorapaxar and TPGS-PLGA loading Vorapaxar to reduced apoptosis and liver damage in atherosclerosis male rats

Adnan M. Jasim[1], Huda F. Hasan[2]

[1]Department of Physiology and Pharmacology, College of Veterinary Medicine, University of AL-Qasim Green , Iraq

[2]Deptartment of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.
Email: Adnan.mansour81@gmail.com; dr.hudaalqaraghuli@yahoo.com

Abstract

Vascular cell apoptosis, an active type of programmed cell death, has crucial role in atherosclerosis, thus promoting the acceleration of acute cardiovascular events. The importance of this work due to threre is no data clarify about using of new protease activated receptor antagonist on apoptotic activity in liver tissue . Our result confirmed the ability of vorapaxar and its nanoparticles asignificant reduction in serum urea and urea concentration after 10 weeks of treatment. The present study confirmed there is highly a significant ($P<0.05$) increase in the percentages of apoptosis in liver tissue after exposed to oxidative stress specially in T2 and T3 as compared to control and other treated groups. Moreover, rats treated with Varapaxar (T4) and PLGA –loading Vorapaxar appear clear prominent reduced of apoptotic cell near from control.

Introduction

Apoptosis an active form of programmed cell death, plays a complete role in atherosclerosis as well as support the acceleration of acute cardiovascular events (1). there are many pathophysiological properties of apoptotic cells such as shrinkage of cell membrane, active membrane blebbing, and thickening of cytoplasm, cellular fragmentation followed by macrophages was apoptotic bodies engulfment, in the absence of an inflammatory response initiation (2). There are three basic protein families has crucial role in the arrangement apoptosis of the cell : , B-cell .

lymphoma-2 (BCL-2), caspases and IAPs(3) (4). There are two main apoptotic pathways include the extrinsic pathway and intrinsic pathway (Bcl-2 protein family). Both pathways stimulate the cascade of caspase , resulting in cell apoptosis (oxLDL: oxidized low-density lipoprotein; FasL: Fas ligand; TNF: tumor necrosis factor; TNFR: TNF receptor). The pathway type extrinsic interest in membrane-linked death receptors of the tumor necrosis factor -receptor (TNF-R) superfamily, like Fas/CD95, TNF-R1, and the death receptors DR3, DR4 and DR5 that facilitates the activation of procaspase 8 and proteolytic activation of the effector caspases 3, 6 and 7 (1, 5,

6) . Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) screening has been developing to identified apoptotic cells that undergo degradation of vast DNA through the final steps of apoptosis. The method is depened on the capacity of TdT to label blunt ends of double-stranded DNA breaks independent of a template (7). TUNEL fluorescence procedure is a well-confirmed, quick , and easy nonradioactive technique to identify cell undergoing apoptosis. It check free 3′-OH termini in single-stranded breaks in high-molecular-weight nuclear DNA fragments (8). The existence of these groups is accounted a proven marker of apoptosis. This assay allow the dissipation of cell death by apoptosis from necrosis figure (2-20) (9). The relationship between apoptosis and atherosclerotic plaques can be listed by that "apoptosis plays an essential and critical role during the development of atherosclerosis from atherogenesis to atherosclerotic plaque formation, progression and eventually rupture] (10). oxidized LDL its chemical modified lipoprotein start atherosclerotic lesions which trigger vascular cells apoptosis (11)by mean of both activations of caspases (12), thus suppression of the nuclear transcription factor NF-KB (13).commonly types of vascular cells undergo apoptosis (macrophages, VSMCs, T-lymphocytes and endothelial cells,) (14)" . In prosperity, endothelial (E C) cells in atherosclerotic plaques are distinguished by elevating apoptotic rate, resulting to erosion of endothelium and dysfunction. subsequently, adhereance of monocytes to the endothelium and transmigrate to the intima, following expressions of adhesion molecules, as macrophage chemotactic protein-1 (MCP-1) and intracellular cell-adhesion molecule-1 (ICAM-1) (15).apoptosis clarify a greater mechanism accountable for the arrangement of the cellularity of the aorta wall during atherogenesis (16).In developing atheroma, during acute vascular syndromes, massive apoptosis of vascular cells may weaken the fibrous cap, support thrombosis and elevating the risk of plaque perturbation (1).

Materials and Methods

PLGA 50:50 (PURASORB PDLG 5010), Vitamin E TPGS NF Grade and Vorapaxar were purchased from medical express chemistry (MEC, USA). Nanoparticles were prepared by the nanoprecipitation method according to (17) with some modification in the organic phase as well as percentages of organic solvent so as to choose perfect organic phase and concentration result small nanosize and high encapsulant efficiency. Immunehistochemistry abcam/UK with kit code abcam 66108 , this Tunel-based detection kit utilizes Terminal deoxyribonucleotidyl Transferase (TdT) to catalyze incorporation of fluorescein -12-dUTP at the free 3'-hydroxyl ends of the fragmented DNA. In situ direct DNA fragmentation Assay (TUNEL-based detection Kit) .

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is assay for identified of fragmentation of DNA by labeling the 3′- hydroxyl termini in the double-strand DNA breaks created through apoptosis according to (18).

Induction of atherosclerosis

Fifty-five adult male rats were given hydrogen peroxide 0.5% in drinking water and a high cholesterol fed diet (1.50% cholesterol) daily for three weeks. In addition, a single dose of triton 10mg/kg was added at end of the last three weeks of the experiment (19) . After the end of the periods of induction, histopathological sections were done in five animals taken randomly from

each experiment. Furthermore, levels of cholesterol were estimated to ensure the disease was induced Experimental design: Fifty rats were randomly divided into five equal groups. First group (T1): animals in this group were still normal without any treatment as a negative control. Second group (T2): animals in this group were induced with atherosclerosis and treated with distilled water as a positive group. Third group (T3): animals in this group were induced with atherosclerosis and treated with a daily dose of 1.1mg/kg empty nanoparticles given orally. Fourth group (T4): animals in this group were induced with atherosclerosis and treated with a daily dose of 0.38mg/kg of ordinary Vorapaxar given orally. Fifth group (T5): animals in this group were induced with atherosclerosis and treated with nanoparticles loaded with Vorapaxar at a daily dose of 0.11mg/kg given orally by stomach tube. Finally, the experiment was carried out for 10 weeks.

Result and discussion

Liver tissue apoptotic cell:

The present study in table (1) and figure showed there is highly a significant ($P<0.05$) increase in the percentages of apoptosis cell in aorta tissue after exposed to oxidative stress mixed with high fat diet as well as limited dose of triton in among rated group as compared to control . atherosclerosis rats in T2 and T3 that showed a highly significant ($P<0.05$) elevation in apoptotic cell than compared to control and other treated groups to recorded mean value (13.80±1.7 and 11.60±1.5) respectively. Moreover animal treated group with Varapaxar (T4) reveal a significant reduction in the percent of apoptotic cell to become near from (T5) but still a highly a significant($P<0.05$) when compared with control to record mean value (7.40±2.8). The present study confirms reduction in liver tissue of apoptotic cell after treating with PLGA –loading Vorapaxar to reach mean value (5.20±2.5) that still has no a significant difference when compared with control. Urea (mg/dl) and Creatinine (mg/dl) in T2 and T4 record a significant increment ($P<0.05$) in mean values 56.92±6.51, 52.72±6.36 and 1.16±0.097, 1.20±0.094 respectively as compared with all other groups and control treated group. While the Urea and Creatinine in T4 and T5 record no significant variation ($P<0.05$) in mean values 38.30±3.15, 40.55±4.0 and 0.71±0.06, 0.64±0.04 as compared with the normal rats (T1).

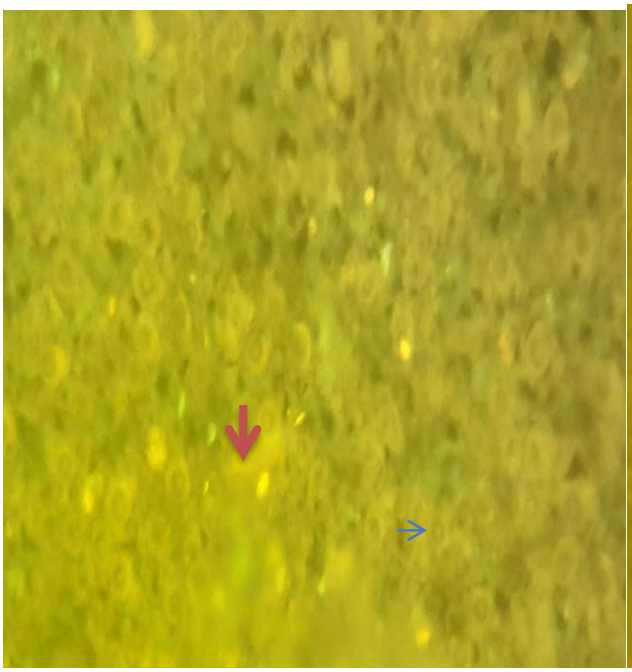
Table (2): The percent of apoptosis,cell in liver tissue of different groups control, positive atherosclerosis, atherosclerotic treated with Vorapaxar, atherosclerotic treated with PLGA –Vorapaxar nanoparticle and atherosclerotic treated with PLGA nanoparticle empty.

Treated groups	T1	T2	T3	T4	T5	LSD
Apoptosis cell %	4.20±0.59 C	12.80±1.8 A	11.60±1.3 A	7.50±2.8 a B	5.20±1.5 a C	3.27
Urea mg/dl	31.41±1.94 B	56.92±6.51 A	52.72±6.36 A	38.30±3.15 B	40.55±4.06 B	11.33
Creatinin mg/dl	0.60±0.01 B	1.16±0.097 A	1.20±0.094 A	0.71±0.06 B	0.64±0.041 B	0.52

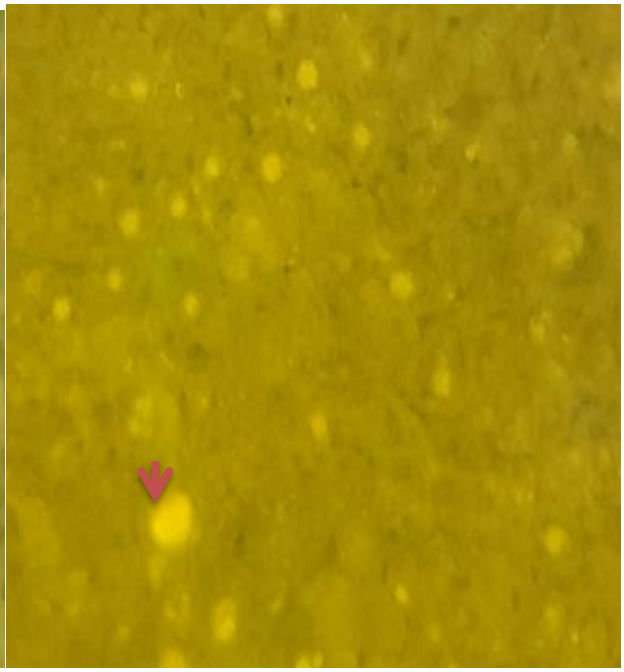
- The value represents mean ±SE
- Different capital letters indicate significant ($P<0.05$) among groups.

Different small letters indicate significant ($P<0.05$) among groups

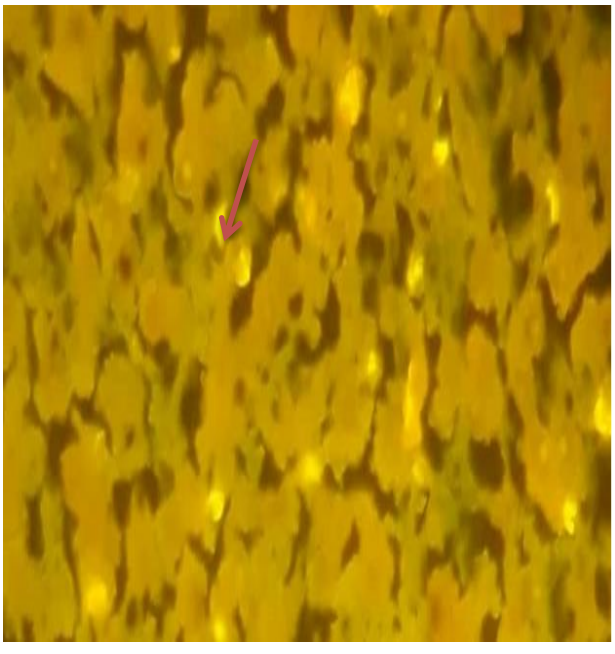
Our data about the reduction of creatinine was agreement with Waasdorp, Duitman (20) noted that administrated vorapaxar to mice induced diabetes by streptozocin was reduced albumin in diabetic mice, as well as diminished plasma cystatin C in in diabetic mice when compared with that, received streptozocin alone. vorapaxar significantly reduced the number of glomeruli with mesangial expansion and reduced fibronectin deposition in diabetic mic when compared with streptozocin , that gives aguide to high protection of kidney from damaged of streptozocin (20) . Our result in table and diagram confirm the ability of vorapaxar to a significant reduction in serum urea concentration in (T4) and (T5) groups after 10 weeks of treatment and there is no significant ($P<0.05$) difference between them from the side and with control from another side. Urea activated intracellular ROS production, thus increases carbamylation and carbonylation of intracellular proteins that promotion alter the function of cytosolic, nuclear, and mitochondrial proteins responsible for the regulation of ROS production (21). Several studies confirm a direct relationship between protein carbonylation levels and atherosclerosis (22, 23). antioxidant properties of vorapaxar potentiate of clear improvement of function to clear the body from waste product and resist oxidative stress result from hydrogen peroxide and a high- fat diet , thus clear prominent in lipid profile will aid the body to restore body function that support liver metabolism and kidney clearance .



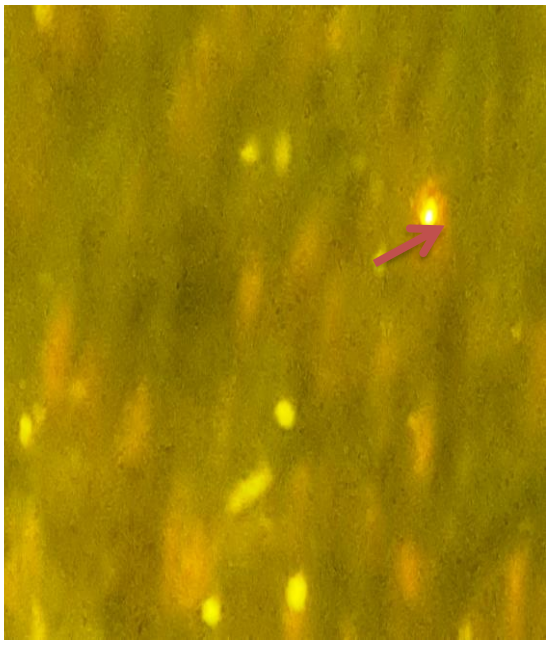
Immunehistological section of liver tissue from (T1) to apoptotic cell , refer apoptosis (X 20)



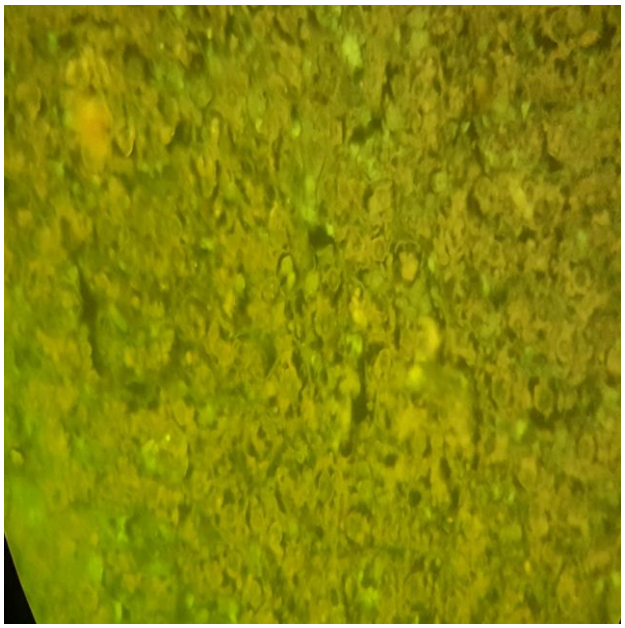
Immune histological section of liver tissue of the induced atherosclerosis rats of (T2) stained by TUNEL X (40) .



Immuehistopathological section of liver tissue from(T3) group stained by TUNEL .



Immuehistopathological section of liver tissue from(T4) group stained by TUNEL .



Immun- histopathological section of liver tissue from(T5) group stained by TUNEL .

The present study confirmed there is highly a significant ($P<0.05$) increase in the percentages of apoptosis, cell in liver tissue after exposed to oxidative stress specially in T2 and T3 as compared to control and other treated groups . Moreover, animal treated group with Varapaxar (T4) reveal a significant reduction in the percent of apoptotic cell while PLGA –loading Vorapaxar appear clear prominent reduced of apoptotic cell near from control . Our result was agreement with Zhang, Okada (24) that reported oxidative stress can induce DNA damage like DNA fragmentation and apoptosis. Hardy, El-Assad (25) confirmed that high-fat diet overexposure elevate the expression of pro-inflammatory cytokines reduce insulin signaling and prompt apoptosis recognized by ER impairments and oxidative stress.

The principle mechanism of apoptosis include eliminated of supererogatory harmful cells (26). conclude that the DNA fragmentation that occurs during apoptosis not only can result cell-autonomously from caspase-activated DNase activity but can also be attributed to a lysosomal acid DNase. Vascular cell apoptosis, an active form of programmed cell death, plays essential role in atherosclerosis and in stent Vascular cell apoptosis, therefore promoting the acceleration of acute cardiovascular events (27). Beyond their antiatherogenic effects, PAR-1 inhibitors, or Vorapaxar, have been shown that effect on an apoptotic process. PAR-1 stimulated by thrombin that highly expressed in exposed to oxidative stress to activate platelet and monocyte recruitment that act to increase vascular LDL oxidized uptake to form foam cell which act synergistically with VSMC to generate atheroma that undergo to foam cell apoptosis to product plaque atherosclerosis, so that Vorapaxar act to prevent this development . The morphological changes during the apoptotic process include cell shrinkage, chromatin condensation, and nuclear and DNA fragmentation (28). Apoptosis plays an essential role in different cardiovascular diseases including cardiac ischemia. This excessive production of ROS in turn activates the apoptotic signaling pathway, leading to cell death . our study showed there is elevating serum TNF-α in atherogenic rats specially that still without treatment , many studies reported that chemokines

such as TNF-α has role in induced apoptosis of cell (29). Waasdorp, Duitman (20) confirmed that Vorapaxar and its nanoparticle inhibited serum TNF-alpha that has potential role in induced apoptosis of ,thus vorapaxar has anti-inflammatory effect was noted by elevating of serum IL10 . Moreover, Vorapaxar has been reported to protect liver tissue from DNA damage was well clear in DNA comet assay technique and immune histopathological section of liver stained by TUNEL . Liver tissue damage is known to be linked with cell apoptosis, created by increased ROS and oxidative stress(30) . for that reason, treatment by PLGAloading Vorapaxar minimize oxidative stress and apoptosis , may be due to long term therapy for 10 weeks aid in return tissue correct arrangement as well as Vorapaxar has in vivo and invitro antioxidant activity was confirmed in this study. Finaly study was concluded that TPGS-PLGA loading Vorapaxar minimize apoptosis in liver and reduced its damage in atherosclerosis male rats .

Reference

1. Katsiki N, Tziomalos K, Chatzizisis Y, Elisaf M, Hatzitolios AI. Effect of HMG-CoA reductase inhibitors on vascular cell apoptosis: beneficial or detrimental? Atherosclerosis. 2010;211(1):9-14.
2. Kavurma M, Bhindi R, Lowe H, Chesterman C, Khachigian L. Vessel wall apoptosis and atherosclerotic plaque instability. Journal of Thrombosis and Haemostasis. 2005;3(3):465-72.
3. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nature reviews Molecular cell biology. 2004;5(11):897.
4. Opferman JT, Kothari A. Anti-apoptotic BCL-2 family members in development. Cell death and differentiation. 2018;25(1):37.
5. Jha K, Shukla M, Kumar M, Shukla VK, Pandey M. Expression of Caspase 3 and Inhibition of Apoptosis Lowers Survival in Breast Cancer. World Journal of Surgical Medical and Radiation Oncology. 2017;6(3).
6. Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. Cell death and differentiation. 2018;25(1):65.
7. Kyrylkova K, Kyryachenko S, Leid M, Kioussi C. Detection of apoptosis by TUNEL assay. Odontogenesis: Springer; 2012. p. 41-7.
8. Ferreira A, Afreen S. Methods related to studying tau fragmentation. Methods in cell biology. 141: Elsevier; 2017. p. 245-58.
9. Sharma R, Masaki J, Agarwal A. Sperm DNA fragmentation analysis using the TUNEL assay. Spermatogenesis: Springer; 2013. p. 121-36.
10. Rossi ML, Marziliano N, Merlini PA, Bramucci E, Canosi U, Belli G, et al. Different quantitative apoptotic traits in coronary atherosclerotic plaques from patients with stable angina pectoris and acute coronary syndromes. Circulation. 2004;110(13):1767-73.
11. Badimon L, Vilahur G. LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: inflammatory resolution versus thrombotic chaos. Annals of the New York Academy of Sciences. 2012;1254(1):18-32.
12. Harada-Shiba M, Kinoshita M, Kamido H, Shimokado K. Oxidized low density lipoprotein induces apoptosis in cultured human umbilical vein endothelial cells by common and unique mechanisms. Journal of Biological Chemistry. 1998;273(16):9681-7.
13. Heermeier K, Leicht W, Palmetshofer A, Ullrich M, Wanner C, Galle J. Oxidized LDL suppresses NF-κB and overcomes protection from apoptosis in activated endothelial cells. Journal of the American Society of Nephrology. 2001;12(3):456-63.
14. Kockx MM, Herman AG. Apoptosis in atherosclerosis: beneficial or detrimental? Cardiovascular research. 2000;45(3):736-46.

15. Hashizume M, Mihara M. Blockade of IL-6 and TNF-α inhibited oxLDL-induced production of MCP-1 via scavenger receptor induction. European journal of pharmacology. 2012;689(1-3):249-54.
16. Geng Y-J, Libby P. Progression of atheroma: a struggle between death and procreation. Arteriosclerosis, thrombosis, and vascular biology. 2002;22(9):1370-80.
17. Rafiei P, Haddadi A. Docetaxel-loaded PLGA and PLGA-PEG nanoparticles for intravenous application: pharmacokinetics and biodistribution profile. International journal of nanomedicine. 2017;12:935.
18. LOZANO GM, BEJARANO I, ESPINO J, GONZÁLEZ D, ORTIZ Á, GARCÍA JF, et al. Density gradient capacitation is the most suitable method to improve fertilization and to reduce DNA fragmentation positive spermatozoa of infertile men. Anatolian Journal of Obstetrics & Gynecology. 2009;1(3).
19. Pashaie B, Hobbenaghi R, Malekinejad H, editors. Anti-atherosclerotic effect of Cynodon dactylon extract on experimentally induced hypercholesterolemia in rats. Veterinary Research Forum; 2017: Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
20. Waasdorp M, Duitman J, Florquin S, Spek CA. Vorapaxar treatment reduces mesangial expansion in streptozotocin-induced diabetic nephropathy in mice. Oncotarget. 2018;9(31):21655.
21. D'Apolito M, Colia AL, Lasalvia M, Capozzi V, Falcone MP, Pettoello-Mantovani M, et al. Urea-induced ROS accelerate senescence in endothelial progenitor cells. Atherosclerosis. 2017;263:127-36.
22. Koeth RA, Kalantar-Zadeh K, Wang Z, Fu X, Tang WW, Hazen SL. Protein carbamylation predicts mortality in ESRD. Journal of the American Society of Nephrology. 2013;24(5):853-61.
23. Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Hörkkö S, Barnard J, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nature medicine. 2007;13(10):1176.
24. Zhang D, Okada S, Yu Y, Zheng P, Yamaguchi R, Kasai H. Vitamin E inhibits apoptosis, DNA modification, and cancer incidence induced by iron-mediated peroxidation in Wistar rat kidney. Cancer Research. 1997;57(12):2410-4.
25. Hardy S, El-Assad W, Przybytkowski E, Joly E, Prentki M, Langelier Y. Saturated fatty acids induced apoptosis in MDA-MB-231 breast cancer cells: a role for cardiolipin. Journal of Biological Chemistry. 2003.
26. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. science. 1998;281(5381):1305-8.
27. Bell CW, Jiang W, Reich III CF, Pisetsky DS. The extracellular release of HMGB1 during apoptotic cell death. American Journal of Physiology-Cell Physiology. 2006;291(6):C1318-C25.
28. Doonan F, Cotter TG. Morphological assessment of apoptosis. Methods. 2008;44(3):200-4.
29. Canault M, Peiretti F, Kopp F, Bonardo B, Bonzi M-F, Coudeyre J-C, et al. The TNF alpha converting enzyme (TACE/ADAM17) is expressed in the atherosclerotic lesions of apolipoprotein E-deficient mice: possible contribution to elevated plasma levels of soluble TNF alpha receptors. Atherosclerosis. 2006;187(1):82-91.
30. Yildar M, Aksit H, Korkut O, Ozyigit MO, Sunay B, Seyrek K. Protective effect of 2-aminoethyl diphenylborinate on acute ischemia–reperfusion injury in the rat kidney. journal of surgical research. 2014;187(2):683-9.