RESEARCH ARTICLE

N- stearoylethanolamine exerts cardioprotective effects in old rats.

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Abstract: Aims: Study of the cardioprotective effects of C18:0 NAE-N-stearoylethanolamine (NSE) in aged rats.

Background: Aging is associated with the slowing down of metabolic processes, diminished physiological processes, changes in hormonal activity, increasing exposure to oxidative stress factors, and chronic inflammation. The endocannabinoid system (ECS) is a major signaling network that plays a pro-homeostatic role in the central and peripheral organs of the human body. A class of minor lipids - N-acylethanolamines (NAEs), which do not activate cannabinoid receptors, except for anandamide, but can potentiate the action of endocannabinoids and have a wide spectrum of biological activity and significant adaptogenic potential, belongs to ECS. The results of different studies over the past decades have established the NAE's protective effect on many pathological conditions.

Objective: In these studies, we focused on investigating the effects of C18:0 NAE N-stearoylethanolamine (NSE) on the intensity of oxidative/nitrosative stress, antioxidant potential, lipoprotein profile and inflammation markers of blood plasma, phospholipid composition and age-related morphological changes of old rat heart tissues.

Methods: The study was conducted on Sprague Dawley male laboratory rats. The three groups of rats were involves in the study design. The first group consisted of young rats aged 4 months (n=10). The second (n=10) and third (n=10) groups included of old rats aged of 18 months. Rats from the third group were administered a per os aqueous suspension of NSE at a dose of 50 mg/kg of body weight daily for 10 days. All groups of rat rats were kept on a standard vivarium diet. The blood plasma, serum, and heart of rats were used for biochemical and histological analysis.

Results: The cardioprotective effect of N-stearoylethanolamine in old rats was established, which was expressed in the normalization of the antioxidant system condition and the level of proinflammatory

cytokines, positive modulation of blood plasma lipoprotein profile, normalization of heart tissue lipid composition and significant reduction of age-related myocardium morphological changes.

Conclusion: The revealed effects of N-stearoylethanolamine can become the basis for developing a new drug for use in complex therapy to improve the quality of life of older people.

Keywords: N-stearoylethanolamine, aging, antioxidant enzymes, lipid peroxidation, proinflammatory cytokines, nitric oxide, phospholipids, cholesterol, rats

1. INTRODUCTION

Aging is one of the most urgent medical and social problems today. Despite progress in treating cardiovascular disease, the overall prevalence of age-related diseases, including cardiovascular disease, is still increasing and is a significant cause of chronic disability, morbidity, and mortality among older adults [1, 2].

Over time, the functioning of the immune system decreases in the body, which, as a rule, is combined with a low-grade inflammatory state. This state of local or generalized chronic inflammation (which is accompanied by the typical phenomena of cellular aging such as telomere loss, oxidative stress, and DNA defects) damages all organs, leading over time to the development of age-related diseases such as osteoporosis, osteoarthritis, atherosclerosis, neurodegenerative disorders, and cancer. Recently, there has been increasing evidence in the literature that growth levels of the proinflammatory cytokines IL1β, IL-6, TNFα and defects in innate and adaptive immunity are likely key mechanisms by which chronic biological, chemical, and psychological stressors can have powerful effects on people's health, accelerating the risk of age-related diseases [3, 4, 5]. There is also growing evidence that controlling inflammation can increase the chances of "healthy" aging [6].

Recently, it has been established that the endocannabinoid regulatory system (ECS) plays a significant role in aging [7]. Intensive study of the body's ECS over the last decade has provided a large amount of data on the critical role of ECS in many pathological conditions, including type 2 diabetes, metabolic syndrome, cancer, brain injuries, and posttraumatic conditions [8, 9]. The role of the ECS in the neuro- and immunomodulation described above means that in addition to CB-receptor agonists such as tetrahydrocannabinol and WIN55,212-2, other components of the ECS may represent suitable targets for the development of new therapeutic drugs. The endogenous ligands of cannabinoid receptors are 2-arachidonoylglicerol and N-arachidonoylethanolamide, also named anandamide. ECS also contains cannabinoid-like compounds - a class of minor lipids - N-acylethanolamines (NAEs), have a wide spectrum of biological activity and significant adaptogenic potential [10]. Even though NAEs (except anandamide) do not bind directly to cannabinoid receptors [11], they may affect this system through an entourage effect [12], meaning they enhance endocannabinoid signaling and share enzymatic pathways with anandamide [13].

The protective effect of NAEs with saturated acyl chain for different pathological conditions, such as ischemia-reperfusion damage to organs, the intoxication of various genesis, diabetes mellitus type I and insulin resistance, allergic reactions, and inflammatory processes, have been established in a series of studies in recent decades [14-25].

The main aim of this study was the investigation of the cardioprotective effect of NSE with aging.

There were 5 objectives for this study: 1) studying the effect of C18:0 NAE - N-stearoylethanolamine (NSE) on the intensity of oxidative/nitrosative stress and the antioxidant potential of blood plasma, 2) studying the effect of NSE on the lipoprotein profile, 3) studying the effect of NSE on the markers of inflammation in blood plasma, 4) studying the effect of NSE on the phospholipid composition and 5) studying the effect of NSE on the age-related morphological changes of heart tissues in aging rats.

2. MATERIALS AND METHODS

2.1. Study design

The research was conducted on Sprague Dawley male rats in the period from July 2022 to October 2022. Rats were selected as research subjects because short life expectancy and availability sufficient quantity of biological material for studying. The rats were kindly provided by the vivarium of the Institute of Pharmacology and Toxicology of the National Academy

of Sciences of Ukraine. The first group consisted of young rats aged 4 months (average weight 432 ± 12 g; n=10). The second (n=10) and third (n=10) groups included of old rats aged of 18 months (average weight 503 ± 11 g). Rats from the third group were administered a per os aqueous suspension of NSE at a dose of 50 mg/kg of body weight daily for 10 days (the final average body weight was 508 ± 8 g). All groups of rat remained on a standard vivarium diet.

A sample size of 10 animals on each group was calculated to provide a 95% level of confidence.

All animal experiments were conducted with humane and customary care and followed a protocol approved by the internal Institutional Animal Care and Use Committee. All rats were housed in a barrier facility with a 12-hour light/12-hour dark cycle. Animals were removed from the experiment under CO_2 anesthesia [26].

Further studies were carried out using blood plasma and rat heart homogenates. The lipid composition and morphological state of heart tissue were studied. The lipoprotein profile and level of proinflammatory cytokines were studied in blood plasma of rats.

2.2. Lipid analysis

The extraction of lipids from rat heart tissues was carried out according to the method of Blight and Dyer [27]. Lipid extracts (in benzene) were stored in a refrigerator at -18 °C.

Separation of phospholipids was carried out by two-dimensional micro-thin-layer chromatography according to the method of Svetashev and Vaskovsky [28,29] on plates ("Sorbfil", Russia) in solvent systems: chloroform: methanol: benzene: 28% ammonia in the corresponding volume ratio 65:30:10:6-8 (1st direction) and chloroform: methanol: benzene: acetone: glacial acetic acid: water in the ratio 70:30:10:5:4:1 (2nd direction). Phospholipid classes were identified as described in [30]. The content of individual phospholipids was determined by the method of Vaskovsky and Kostecsky [31].

The separation of free and esterified cholesterol fractions was carried out by one-dimensional micro-thin-layer chromatography on plates ("Sorbfil", Russia) in the solvent system hexane: diethyl ether: glacial acetic acid in the corresponding volume ratio of 85:15:1. The free cholesterol zone was removed from the plate, transferred to a microcolumn and washed with diethyl ether. The zone of esterified cholesterol was methylated with 3M HCl in methanol, then extracted with hexane, purified on plates (“Sorbfil”, Russia) in benzene, removed from the plate and eluted with diethyl ether. After evaporation of the solvent (diethyl ether), the dry cholesterol residue was analyzed on a Carlo Erba gas-liquid chromatograph (Italy) with a flame ionization detector on a 0.5 m- glass column with an inner diameter of 3 mm, which was filled with Chimalite W (80- 100 mesh) and saturated with 1.5% liquid phase OV-1 ("Shimadzu", Japan), at injector and detector temperatures of 250 °C and 270 °C, respectively, and a programmed temperature of 180-250 °C (100 C/min). Cholesterol quantification was carried out according to the calibration curve of the cholesterol standard.

2.3. Determination of lipid peroxidation markers

Lipid peroxidation was assessed as thiobarbituric acid reactive substances formed as measured by the thiobarbituric acid technique, as described in our previous work [32].

2.4. Determination of antioxidant enzyme activity

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by the nitro blue tetrazolium reduction level in the presence of NADH and phenazine methosulfate [33]. The activity of catalase (CAT, EC 1.11.1.6) in rat myocardium and blood plasma was determined by the level of hydrogen peroxide degradation according to the Koroliuk method [32]. Glutathione peroxidase (GP, EC 1.11.1.9) activity was measured by the accumulation of oxidized glutathione in the incubation [34].

2.5. Determination of lipoprotein profile

The content of triacylglycerols, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) in the blood of rats was determined by the colorimetric method using a commercial kit ("Filisit-Diagnostics", Ukraine).

2.6. Cytokine assay

The content of the cytokines IL1-beta and TNFα in the blood of rats were measured by the immunoenzymatic method using the commercial kits "Invitrogen Rat IL1beta ELISA Kit" and "Invitrogen Rat

TNF alpha ELISA Kit" ("Thermo Fisher Scientific", Austria).

2.7. Nitric oxide content assay

The nitrite anion content was determined according to a previously described method [35].

2.8. Protein content determination

The concentration of total protein was measured by the Bradford method [36].

2.9. Morphological studies

Morphological studies of the rat heart were carried out using standard histological methods [37]. 10% buffered formalin, ethanol, and paraffin type 6 (Richard-Allan Scientific, USA) were used to prepare histological preparations. Histological sections were made using a MICROM HM325 microtome. The preparations were stained with hematoxylin and eosin, and sections with mast cells were stained with toluidine blue.

Evaluation of each of the rat`s heart histological preparations were carried out by two independent researchers.

2.10. Statistical analysis

All data are presented as the mean ± SEM. Student's t-test was used and $P < 0.05$ was considered statistically significant.

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3. RESULTS AND DISCUSSION

3.1 Effect of NSE on the intensity of oxidative/nitrosative stress, antioxidant potential and the content of inflammatory markers in the blood plasma of aging rats

According to the extended free-radical theory of aging, the critical marker of the aging process is the increase in free-radical compound formation against the background of a decrease in antioxidant enzyme activity [38,39].

It has been shown that increasing the activity of both CuZn-SOD and CAT significantly prolongs the maximum life span, and decreasing the expression of SOD in various species reinforces their vulnerability to oxidative stress [40]. Catalase is one of the antioxidant enzymes that considerably alleviates oxidative stress by breaking down cellular peroxide. CAT deficiency or dysfunction is believed to be associated with the pathogenesis of many age-related degenerative diseases, such as diabetes, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia [41]. In contrast, it was shown in transgenic mice models that CAT overexpression increases life expectancy by delaying the onset of cardiac pathologies, cataracts, and oxidative stress [42]. Glutathione peroxidase GPX1 was the first identified mammalian selenoprotein. This antioxidant enzyme detoxifies intracellular hydrogen peroxide (H_2O_2) [43]. It was shown that with aging and the increase in oxidized proteins accumulation, the antioxidant selenoproteins expression decreases, thus reducing their overall antioxidant activity [44]. Blood plasma glutathione peroxidase (GPx) reduces the peroxide content in the blood and plays an essential role in protecting the cardiovascular system, possibly due to nitric oxide level modulation [45].

Fig. (1) shows the plasma SOD, CAT and GPx activity in rats from the two age groups. The diagrams demonstrate that the decrease in the activity of antioxidant enzymes accompanied aging. The administration of NSE to 18-month-old rats for 10 days normalized the activity of all investigated antioxidant enzymes.

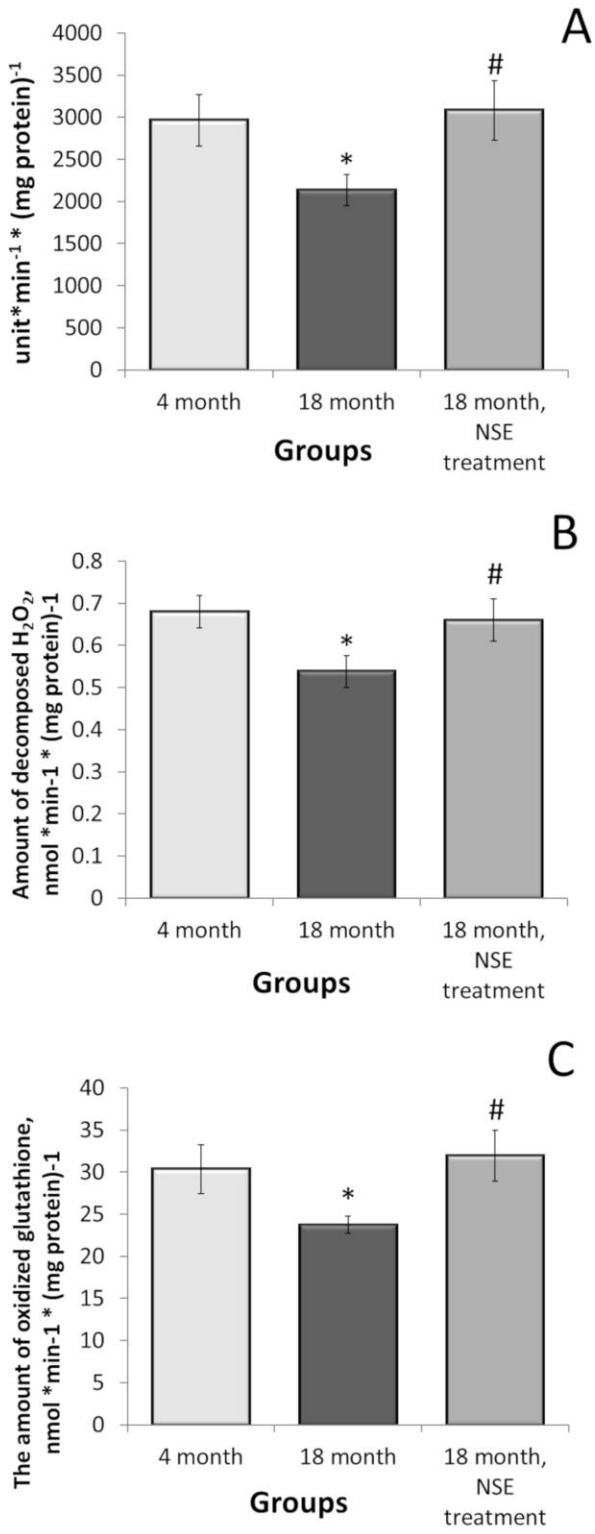

Fig. (1). Plasma activity of superoxide dismutase (A), catalase (B) and glutathione peroxidase (C) in each age group of rats. Data are expressed as Mean ± SD. * represents a statistically significant difference between young rat groups and old rat groups at the significance level of 0.05; # represents a statistically significant difference among age groups and NSE administration at the significance level of 0,05

The free-radical hypothesis of aging suggests that the harmful effects of oxygen-derived radicals are responsible for age-associated functional deterioration in organisms. Since cell membranes contain the structures for producing these radicals, and since these radicals severely damage membranes, it has been suggested that the modification of membrane lipids plays an essential role in the aging process [46]. Decades of research have revealed the complex and multidimensional pathophysiology of aging, which includes increased oxidative stress, redox imbalance, lipid peroxidation, proinflammatory signaling, hyperglycemic stress, and diabetes on the background of mild chronic inflammation and other factors [47]. However, the significant role of peroxidation in developing cardiac dysfunctions during aging remains beyond debate [48].

Our study evaluated the plasma content of thiobarbituric acid reactive substances (TBARS) such as malondialdehyde (MDA) as lipid peroxidation markers. Fig. 2A shows the results of determining the content of TBC-reactive products in the blood plasma of rats. Fig. 2A demonstrates that the TBARS content in 18-month-old rats was probably higher than in young 4-month-old rats and decreased significantly under the influence of NSE. Thus, restoring the antioxidant defense enzymes' activity under the action of NSE (Fig. 1) contributes to the normalization of the lipid oxidative modification products content (Fig. 2A).

It is well known that nitric oxide (NO), in combination with reactive oxygen species (ROS), forms toxic peroxynitrite, which is involved in free radical damage to macromolecules (nucleic acids, proteins, etc.). In turn, nitrite anion is a stable metabolite of nitric oxide. The content of nitrite anion only partially reflects the intensity of nitric oxide generation but indicates the direction of the process: either in the direction of increase or decrease. As is known, the aging process is accompanied by an increase in the activity of the inducible isoform of NO-synthase [49], which causes excessive production of nitric oxide and its free radical derivatives.

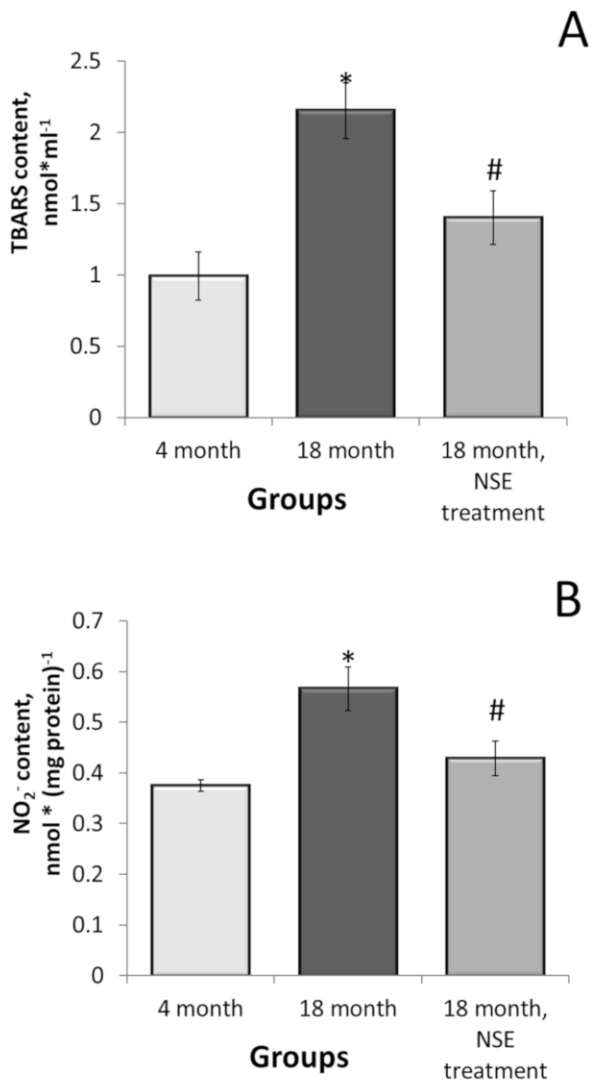

Fig. (2). Plasma content of TBARS (A) and nitrite anion (B) in each age group of rats. Data are expressed as Mean ± SD. * represents a statistically significant difference between young and old rats groups at the significance level of 0.05; # represents a statistically significant difference among age groups and NSE administration at the significance level of 0.05.

It can be seen from Fig.2B that aging is accompanied by a probable increase in the nitrite anion content in the blood plasma of experimental animals, and the use of NSE normalized the indicators in the group of old rats. The obtained data may indirectly indicate a reduction of nitric oxide production under the action of NSE (Fig. 2B).

In previous studies, we showed the NSE's ability to modulate the processes of NO formation by the influence on different NO-synthase isoforms [50, 51]. It is likely that under the conditions of this experiment, the action of NSE is aimed at inhibiting the activity of inducible NO-synthase.

It is known that the NF-κB / iNOS signaling pathway is activated during aging, and the level of NF-κB p105 increases with age [52]. The transcription factor NF-κB regulates the processes of apoptosis and inflammation in the cell. It is usually in the cytoplasm in an inactive state in a complex with the inhibitory I-κB protein. Upon activation by proinflammatory cytokines and ROS, which induce I-κB degradation, NF-κB translocates to the nucleus and binds to DNA to regulate gene transcription. At the same time, in particular, there is the activation of iNOS and a dramatic increase in the production of nitric oxide, which leads to cell death, aging and mortality [53].

Previously, we showed the NSE ability to block the translocation of the NF-κB into the nucleus, which is probably realized through the interaction of NSE with PPARγ [54, 55]. Therefore, it is likely that nitric oxide generation reduction in old rats after NSE administration could also occur via the PPARγ/NF-κB signaling pathway.

It is known that the transcription factor NF-κB is also involved in the regulation of various proinflammatory cytokines production. Fig. 3 shows the content of proinflammatory cytokines IL1β (A) and TNFa (B) in the blood serum of rats.

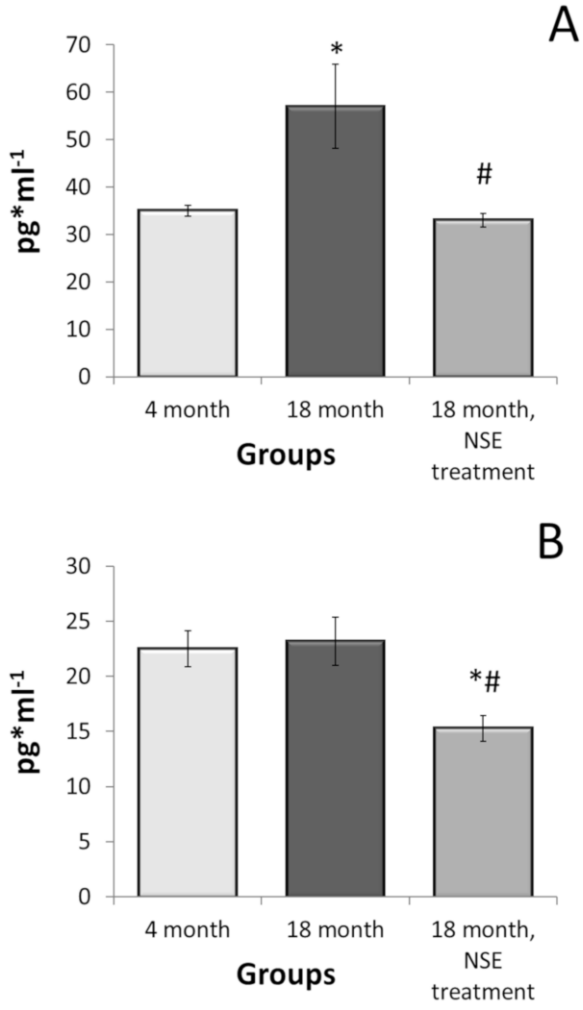

Fig. (3). Serum content of IL1β (A) and TNFα (B) in each age group of rats. Data are expressed as Mean ± SD. * represents a statistically significant difference between young and old rat groups at the significance level of 0.05; # represents a statistically significant difference among age groups and NSE administration at the significance level of 0.05.

Fig. 3 demonstrates that aging was accompanied by a probable increase of IL1β content (Fig. 3A), while the level of another proinflammatory cytokine - TNFα (Fig. 3B), remained unchanged. Administration of NSE to 18-month-old rats normalized and reduced the content of IL1β and TNFα, respectively, in rat blood serum.

Today, more and more attention is paid to the role of chronic low-grade inflammation in the aging process and the pathogenesis of aging-related diseases (dementia, Alzheimer's disease, metabolic syndrome, type 2 diabetes). This chronic inflammation has a multifactorial origin; namely, it can be caused by the aging of the immune system [56], intestine, adipose tissue, etc. [57]. At the same time, chronic inflammation accompanied by the typical phenomena of cellular aging, such as telomere shortening, oxidative stress, and DNA defects, eventually affects all organs and leads to the development of age-related diseases such as osteoporosis, osteoarthritis, atherosclerosis, neurodegenerative diseases, and cancer [58]. So-called "aging" of the endocannabinoid system also occurs: the number of cannabinoid receptors in the central and peripheral nervous system decreases, contributing to the imbalance in the metabolic processes regulation and inflammation control [59].

Fig. 3 shows that NSE normalized the content of IL1-beta and reduced the level of TNFa in the blood serum of old rats. Our previous studies have shown that the effect of NSE on proinflammatory cytokines levels is due to its ability to inhibit the transcriptional activity of NF-kB, which controls the inflammatory response in cells [54]. We obtained preliminary results on the involvement of PPAR-gamma nuclear receptors in realizing the NSE anti-inflammatory effect. [55]. It should be noted that PPARs function as transcription factors and regulate the expression of genes involved in the regulation of lipid metabolism, anti-inflammatory response, etc., and can also indirectly affect the activity of antioxidant enzymes (for example, through the PI3K-Akt signaling pathway [60]), modulate the activity of inducible NO-synthase [61].

Therefore, we assume that the effects of NSE on the antioxidant enzyme activity, nitric oxide production, and proinflammatory cytokines level (Fig. 1-3) under aging conditions can be realized through PPAR-gamma involvement.

3.2. The effect of NSE on the lipoprotein profile and the content of triacylglycerols in the blood of rats during aging

It is known that aging is accompanied by an increase of triacylglycerols concentration and low-density-lipoprotein (LDL) cholesterol in the blood plasma and a decrease in the ability of high-density-lipoproteins (HDL) to remove cellular cholesterol [62].

Studies on rodents have shown that aging leads to cholesterol accumulation in the liver and heart. At the same time, contrary data indicate a decrease in cholesterol content in the heart of rats with aging, regardless of gender [63].

On the other hand, aging is also associated with a cholesterol content reduction in brain structures due to its synthesis decline and the growth of the level of cholesterol 24-hydroxylase (the enzyme responsible for removing cholesterol from the brain) [64, 65].

The lipoprotein profile is an integral indicator of lipid metabolism, and its changes argue for the presence of metabolic disorders. In the conditions of our experiment, a substantial LDL content increase against the background of a significant decrease in HDL level in the blood of rats indicates a lipid metabolism violation, primarily in the liver tissue and the blood atherogenic potential growth. A decrease in HDL cholesterol content demonstrates a cholesterol removal ability decline [66].

Fig. 4 shows the content of LDL cholesterol (A), HDL cholesterol (B), and triacylglycerols – TAG (C) in the blood of rats.

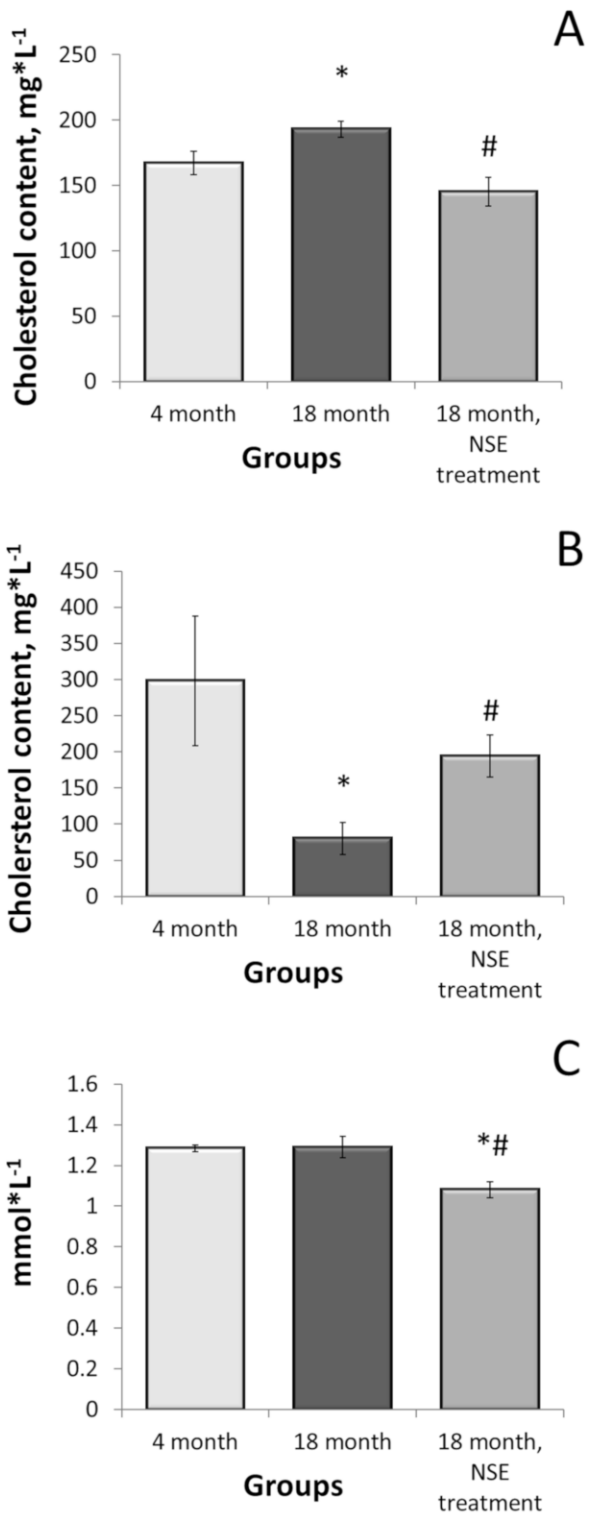

Fig. (4). Blood content of LDL cholesterol (A), HDL cholesterol (B), and TAG (C) in each age group of rats. Data are expressed as Mean ± SD. * represents a statistically significant difference between young and old rats groups at the significance level of 0.05; # represents a statistically significant difference among age groups and NSE administration at the significance level of 0.05.

Fig. 4 demonstrates that LDL cholesterol content is probably higher, and HDL cholesterol content is significantly lower in old rats than their corresponding values in young rats. The TAG level in the blood serum had no significant changes in different age rats.

NSE oral administration led to the normalization of both LDL (Fig. 4A) and HDL (Fig. 4B) cholesterol ratios in the blood of 18-month-old rats, as well as a probable decrease in the TAG content (Fig. 4C). The obtained results indicate NSE antiatherogenic effect.

Our previous studies on a model of experimental dietary-induced insulin resistance in rats showed that NSE also normalized the LDL and HDL cholesterol content [67]. We hypothesize that this NSE effect is related to its hepatoprotective properties, which we described earlier [68].

Fig. 5 illustrates the cholesterol content in rat hearts.

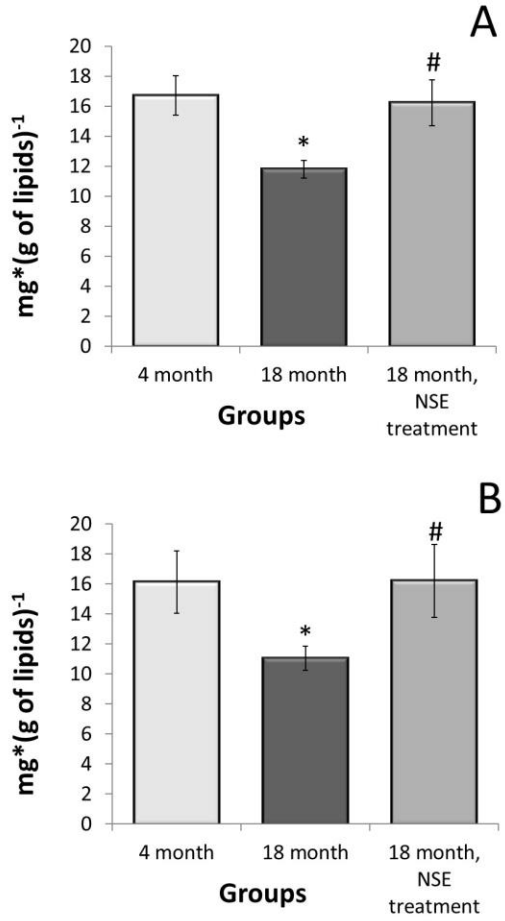

Fig. (5). Heart content of total cholesterol (A) and free cholesterol (B) in each age group of rats. Data are expressed as Mean ± SD. * represents a statistically significant difference between young and old rat groups at the significance level of 0.05; # represents a statistically significant difference among age groups and NSE administration at the significance level of 0.05.

Fig. 5 shows that the content of total (Fig5A, bar 2nd) and free (Fig5B, bar 2nd) cholesterol levels in old rat hearts were probably lower than appropriate figures in the control group. This data is entirely consistent with the data of other researchers who observed a heart cholesterol content decrease during aging [63]. The heart content of both total cholesterol (Fig5A, bar 3rd) and free cholesterol (Fig5B, bar 3rd) in old rats treated with NSE probably did not differ from its content in the hearts of control animals. That is, NSE contributed to heart cholesterol level normalization in old rats.

Today, it is well known that PPARs play an essential role in lipid metabolism. PPAR-alpha and PPAR-gamma activators induce the expression of the gene encoding ABCA1, also known as the cholesterol efflux regulatory protein (CERP) [69]. This protein functions as a cholesterol efflux pump in the cellular lipid removal pathway. It also mediates the efflux of cholesterol and phospholipids to lipid-poor apolipoproteins (apoA1 and apoE) (reverse cholesterol transport), which then form nascent high-density lipoproteins (HDL). PPAR expression and activity have been shown to decrease in some tissues, including the liver and heart of aging rodents [70]. Such PPAR activity decline may result from increased oxidative stress and contribute to the development of age-related dyslipidemia [71].

Given the ability of NSE to activate PPARγ, this may be one of the possible mechanisms [54, 55] of its normalizing effect on cholesterol content in heart cells of old rats. However, this needs further research.

3.3. NSE effect on age-related phospholipid composition changes of rat myocardium

Fig. 6. shows the phospholipid composition of rat myocardium.

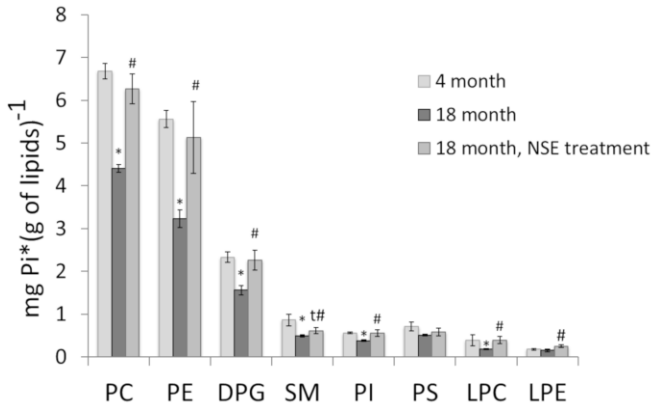

Fig. (6). Heart content of phospholipids in each age group of rats. Data are expressed as Mean ± SD. PC, phosphatidylcholine; SM, sphingomyelin; PS, phosphatidylserine; DPG, diphosphatidylglycerol; PI, diphosphatidylinositol; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; * represents statistically significant difference between young rats groups and old rats groups at the significance level of 0.05; # represents statistically significant difference among age groups and NSE administration at the significance level of 0.05.

Fig. 6 demonstrates that the content of all major phospholipids (PL) in the hearts of 18-month-old rats significantly decreased relative to their values in the 4-month-old rat group.

Such changes are caused by slowing down metabolic processes, especially lipids de novo synthesis. It is also important to pay attention to the decrease in the content of DPG - a distinctive PL of mitochondrial membranes. It may indirectly indicate mitochondrial dysfunction development during aging [72, 73]. A reduction of the content of PLs involved in ensuring membrane fluidity, such as PC, PE and their lysoforms, can indicate an increase in cell membrane rigidity [74] and a decrease in the content of anionic PLs – PI and PS - about some disturbances in cell signaling and transmembrane transport [75].

NSE administration to 18-month-old rats caused a statistically significant growth of all investigated PL content in the heart compared to the indicators of the same-aged rats that did not receive NSE, which indicates the activation of PL de novo synthesis. At the same time, the PL content in old rats' hearts did not differ from its level in the young group. That is, NSE administration normalized the PL composition of cardiomyocyte membranes.

Considering that NSE can act as an agonist of PPAR-gamma receptors, which regulate the expression of the lipogenic genes, such an effect of NSE is possible and requires further detailed studies.

The duration of the effect we discovered is also of interest because, in the conditions of this experiment, we recorded the result of a 10-day administration of NSE to old rats. However, the effects of a single NSE administration remain unexplored.

3.4. Age-related morphological changes in rat heart tissue

Based on the obtained data on the NSE effect on some biochemical characteristics of the rat cardiovascular system during aging, it was also reasonable to investigate NSE influence on heart tissue morphological changes.

General cardiovascular risk factors, coronary heart disease, heart failure, arrhythmias, and cardiomyopathy have a significant prevalence in older individuals and are characterized by peculiar clinical manifestations that have specific features depending on the age of the patients. At the same time, the phenotype of heart aging, both in healthy people and in patients with cardiovascular diseases, reflects the morphological changes occurring in the myocardium at the cellular level [76].

The results of the conducted histological studies showed that structural rearrangements occurred in rat hearts during aging and were possibly aimed at ensuring age-related partially impaired functions. Thus, in the left ventricle myocardium of the heart of old rats, in contrast to young ones, hypertrophy of individual cardiomyocytes and small groups of cells was observed, which occurred against the background of the development of dystrophic-destructive changes in the myocardium. That manifested in the form of granular dystrophy, wavy deformation and contracture damage of cardiomyocytes, as well as their monocellular necrosis and apoptosis (Fig. 1). This was confirmed by an increase in the number of TUNEL-positive ("+") cells in the myocardium of old rats compared to young group ($p<0.05$; Fig. 8), which indicates a growth in cardiomyocytes apoptosis intensity during aging.

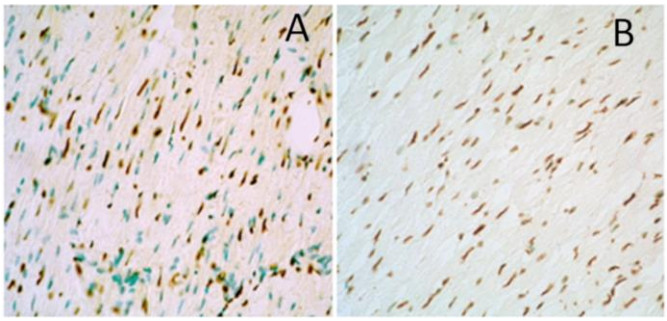

Fig. (7). Morphological characteristics of cell apoptosis intensity in the left ventricle myocardium of the heart in old rats, characterized by quantitative changes in TUNEL-positive cells in the myocardium: (A) – control; (B) – influence of NSE. TUNEL method, x200.

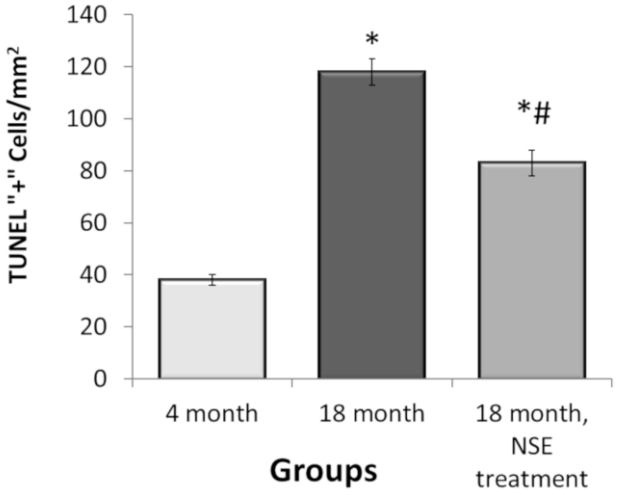

Fig. (8). Changes in the number of TUNEL-positive ("+") cells in the left ventricle myocardium of experimental rats. Data are expressed as Mean ± SD. * represents a statistically significant difference between young rats' groups and old rats' groups at the significance level of 0.05; # represents a statistically significant difference between age groups and NSE administration at the significance level of 0.05

It should also be noted that in old rats, in contrast to the group of young animals, small foci of perivascular edema were often observed in the myocardium, which spread to the adjacent interstitial connective tissue (Fig. 9 A). Small foci of inflammatory lymphocyte and macrophage infiltration were often detected here (Fig. 9 B), where fibroblasts and mast cells, often with signs of their degranulation, were also observed among mononuclear cells (Fig. 9 C), which confirms the development of myocardium low-gradient inflammation during aging.

Under such conditions, in old rats, unlike young ones, diffuse interstitial fibrosis was observed in the myocardium with the formation of clusters of coarse collagen fibers in the extracellular matrix, often with signs of swelling and detachment (Fig. 9 D). Also, in the myocardium of old rats, in contrast to young ones, small caliber arteries with pronounced medial hypertrophy were found to be devastated or filled with formed elements of blood. This phenomenon was probably due to hyperplasia and hypertrophy of smooth muscle cells of the vascular media, as well as significant thickening of the internal elastic membrane, which often was corrugated.

Along with this, the formation of small foci of plasma leakage and sometimes hyalinosis of the media (Fig. 9 A) were observed in some arteries and veins of small caliber. The swelling and dystrophic changes of the endothelium, which forms the intima, also accompanied the abovementioned alterations. At the same time, the orientation of endotheliocyte nuclei perpendicular to the basement membrane was revealed in some arteries and veins of small caliber, which is a characteristic manifestation of the cell reaction in response to hypoxia.

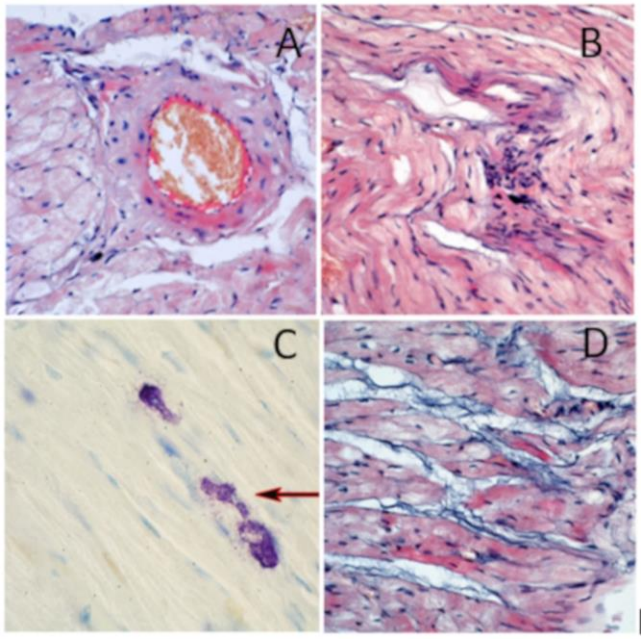

Fig.(9). Representative morphological changes of the myocardium of old rats: (A) – dystrophic changes of cardiomyocytes, pronounced perivascular edema, hypertrophy of the media of a small-caliber artery, plasma leakage and its wall hyalinosis; (B) – cardiomyocytes dystrophic changes and a small focus of inflammatory lymphocyte and macrophage infiltration; (C) – mast cells with

signs of their degranulation in the interstitial connective tissue (←); (D) – diffuse fibrosis, edema and delamination of collagen fibers of the interstitial connective tissue. Staining: (A, B, D) – hematoxylin and eosin, x200; (C) – toluidine blue, x1000.

The results of morphological studies of the hearts of old rats treated with NSE showed that the frequency of morphological signs characterizing the development of inflammatory and dystrophic-destructive changes in the myocardium decreased. In particular, a reduction in the frequency of perivascular and interstitial edema foci and lymphocyte and macrophage infiltration was observed in the myocardium. At the same time, in the cell composition of the inflammatory infiltrate, we noted a decrease in the number of lymphocytes and, accordingly, an increase in the number of macrophages, among which mastocytes were presented. Mastocytes were represented by a pool of mononuclear cells with a cytoplasm densely filled with secretory granules. Mast cells in a state of degranulation, which was characteristic of old rats, were difficult to find (Fig. 10 A). Such changes in mastocytes may indicate the ability of NSE to influence their morphological state and accordingly influence the secretory function of these cells.

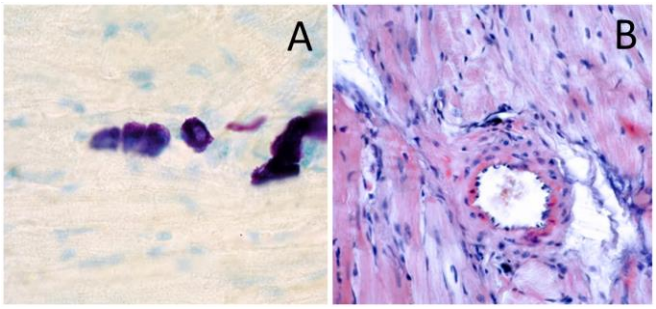

Fig. (10). Morphological changes in the myocardium of old rats after NSE administration: (A) – hyperplasia and hypertrophy of mast cells in the interstitial connective tissue without noticeable signs of their degranulation; (B) – a small-caliber artery with hypertrophy of the media and a small focus of perivascular edema. Staining: (A) – toluidine blue, x1000; (B) – hematoxylin and eosin, x200.

Under the influence of NSE, areas with signs of dystrophic changes in cardiomyocytes, particularly contractile damage and their wavy deformation, were detected in the myocardium less often than in old intact rats. Signs of myocardium blood circulation disorders, such as venous congestion, were also observed less often. At the same time, media hypertrophy due to hyperplasia and smooth muscle cell hypertrophy, which are characteristic of aging, were sometimes detected in the walls of individual small-caliber arteries. Signs of pronounced edema, plasma leakage and hyalinosis in the arteries walls were rarely found or not detected (Fig. 10 B). Thus, indicated NSE's positive influence on the structural and functional state of old rat myocardium should be considered as a cardioprotective effect of NSE. As a result, during the oral administration of NSE, blood circulation processes in the myocardium significantly improve, and the manifestations of inflammatory and dystrophic-destructive changes decrease. A decrease in the frequency of detection of monocellular necrosis and apoptosis of cardiomyocytes in the myocardium of old rats under the influence of NSE confirmed this. At the same time, it was established that NSE could reduce the intensity of cardiomyocyte apoptosis in old rats. It was indicated by a significant ($p<0.05$) reduction in the number of TUNEL-positive cells in the myocardium after NSE administration compared to untreated 18-month-old rats (Fig. 8).

Thus, aging is accompanied by the development of myocardium structural and functional changes, which characterize marked circulatory disorders, the development of low-gradient inflammation, as well as the development of dystrophic-destructive changes in cardiomyocytes.

At the same time, NSE affects the structure and functions of blood vessels and myocardium, which is manifested by a decrease in the blood vessels wall permeability (especially the microcirculatory channel), a reduction of the myocardium inflammatory changes manifestations, as well as dystrophic and destructive changes in cardiomyocytes. It is evidenced by a decrease in the number of TUNEL-positive cells in the myocardium of old rats under the influence of NSE and characterizes apoptosis deceleration.

CONCLUSION

Our findings show that NSE increases the blood plasma antioxidant enzymes activity, decreases the content of IL1-beta and TNFa in the blood serum and restores the lipoproteins level, heart phospholipids content of old rats. NSE exerts a positive influence on the structural and functional state of old rat myocardium that may be considered as a cardioprotective.

The revealed effects of N-stearoylethanolamine can become the basis for developing a new drug for use in complex therapy to improve the quality of life of older people.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the internal Institutional Animal Care and Use Committee.

HUMAN AND ANIMAL RIGHTS

The animals' procedures followed were in accordance with the standards set forth in the eighth edition of "Guide for the Care and Use of Laboratory Animals" published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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