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Activity of oxidative processes in the rats' body of different age, affected by sodium nitrite, on the background of tobacco intoxication

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Abstract
In terms of the progression of man-made pollution one of the priority areas of toxicology and medicine is the study of the characteristics and mechanisms of action of xenobiotics combined activity – risk factors of many ecologically dependent diseases. Among the most common xenobiotics – pesticides, nitrites and nitrates, heavy metals. Tobacco smoke belongs to the most aggressive "oxidative pollutants." It is known that in the basis of pathogenic effect of polluted by pollutants or tobacco smoke air is an oxidant aggression on the mucosa of the respiratory tracts with reactive oxygen species, leading to activation of lipid peroxidation and damage of biological membranes.
The aim of this work was to study the activity of free radical processes in rats of different ages poised with sodium nitrite against tobacco intoxication.
Experiments were performed on immature and mature rats that within 15 days were exposed to tobacco smoke affection. For 24 hours before the end of the experiment the animals of the group I were injected with sodium nitrite at a dose of 45 mg / kg of body weight, in group II – sodium nitrite was administered for 72 hours before euthanasia. Rats were taken out of the experiment under thiopental anesthesia. We use the general principles of experiments on animals approved and consistent with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes when performing the research.
We found the probable increase in the content of reactive oxygen species in blood after poisoning by tobacco smoke leading to activation of lipid peroxidation and oxidative modification of proteins, accumulation of toxic intermediate products in the body and the development of oxidative stress. In all age groups of rats we observed a tendency to increase the content of lipid peroxidation and oxidative modification of proteins in the bodies of animals. Defeat of rats was complicated by application of additional toxicant – sodium nitrite. Immature and old rats were more sensitive to the simultaneous action of tobacco smoke and sodium nitrite, in which the content of TBA-active products and 2.4 dinitrophenylhydrazone significantly exceeded ($r \leq 0.05$) the level of intact control in blood serum, liver, lungs and heart.

Keywords: Tobacco smoke; sodium nitrite, reactive oxygen species, lipid peroxidation, oxidative modification of proteins, oxidative stress

1. Introduction
Cigarette smoking among population is growing every year. Today in the world there is about 1.3 billion people who smoke, in Europe this number is 28%. Smoking threatens human health as acting as one of the strength pollutant not only the human body, but also the environment [1].
Cigarette smoke contains about 1,900 components under the influence of which toxic, mutagenic and carcinogenic effect on human body is possible. In the room where someone smokes concentration of nicotine is from 8 to 20 mg / m^3 of air; and in the room where smoking is prohibited, its concentration is 0.3 mg / m^3 of air. Tobacco smoking is a major cause of diseases origin and premature death from them [2–5].
Significant environmental and biomedical problem in the agro-industrial regions is a combined effect on the body of people and animals of inorganic nitro compounds and other toxic factors accompanied by cases of nitrate-nitrite intoxication [6–7].
The contamination in the body both nitrates and tobacco smoke disturbs the equilibrium in the system of oxidants / antioxidants and leads to the formation of oxidative stress [8–10].
It is known that the toxic action of nitrates and nitrites consists in the ability to activate free radical oxidation processes leading to the development of tumor processes, inhibition of DNA synthesis, enzyme system dysfunction [11–12].

In addition, acute and severe poisoning develops hemic hypoxia, previously considered the main consequence of nitrite intoxication [13]. Herewith, lesions of almost all organs and tissues occur in the body of animals and people.

When smoking generation of reactive oxygen species (ROS: O^{2}-, O^{21}, OH^{-}, $H_2O_2^{-}$, etc.) occurs, which plays an important role in many physiological and biochemical processes. Under the influence of extreme factors of various origins (chemical contamination, ionizing radiation, hyper- and hypoxia, toxins, inflammation) formation of ROS in living organisms are enhanced, leading to the development of oxidative stress in the body accompanying by activation of processes of lipid peroxidation and oxidative modification of proteins [14–15].

In real life, people are often exposed to more toxic factors leading to the general poisoning of the body and involving in the process the damage of many organs.

In literature data there are no research results of metabolic disorders in animals under conditions of combined influence of nitrites and tobacco smoke, which caused the interest in connection with the spread of these toxins in the environment.

The aim of the work was the study of the activity of free radical processes in the rats' body of different age groups poisoned by sodium nitrite on the background of tobacco smoke affection.

2. Material and Methods

White mongrel male rats that were kept on a standard diet at vivarium of Ternopil State Medical University were used for the experiment. The rats were divided into two age groups: I – immature rats weighing 60–80 g, II – mature rats weighing 180–200 g. Each age group consisted of two subgroups – intact control and experimental group. Rats of the research groups within 15 days were exposed to tobacco smoke. The test animals were divided into 3 groups. One of them for 24 hours before the end of the experiment was injected sodium nitrite at a dose of 45 mg / kg, the second – sodium nitrite was administered for 72 hours before euthanasia. The third group of rats was exposed to the toxic effect of only tobacco smoke. Dependence model from chronic action of tobacco smoke was created using a sealed chamber in a volume of 30 liters, allowing to fumigate animals in free behavior. Tobacco smoke formed by combustion of 6 cigarettes Prima Silver (blue) and (containing 0.6 mg of nicotine and 8mg of tar), through openings in the chamber was fed into it. 6 animals both within 6 minutes were simultaneously in the experiment chamber. Animals in the control group also were during 6 minutes in the sealed chamber, but were not exposed to tobacco smoke.

After 15 days from the beginning of the affection by tobacco smoke the animals were deduced from the experiment by euthanasia with thiopental anesthesia.

Blood, serum blood, liver, lungs and the myocardium of animals were harvested. 10% homogenate on isotonic solution was prepared from the experimental tissues.

The content of reactive oxygen species (ROS) were determined in blood neutrophils by the method [16–17]. Population of blood neutrophils was obtained by centrifugation on a double density gradient 1.077 and 1.093 ficoll-verografin. After 40 minutes of centrifugation at 4 C and speed of 1500 rev / minute two interphase were formed. Upper interphase (on the verge of plasma-verificoll in density of 1.077) consisted of mononuclear cells – 80% of lymphocytes, 15–18% of monocytes and low (2–3%) addition of granulocytes. Lower interphase (on the verge of gradient solutions of density in 1.077–1.092) was the population of neutrophils at 98–100%. Cell viability in a test with trypan blue was 98–99%. Analysis of cell samples to determine the ROS neutrophils was conducted on the current cytometer Epics XL (Beckman Coulter, USA) using 2.7–dihydrodichlorofluorescein diacetate. The value of the investigated parameter was expressed in percentages (intensity of luminescence per cell). Activity of lipid peroxidation processes was evaluated on the content of TBA – active products (TBA – AP) [18], the intensity of oxidative modification of proteins containing 2.4 dinitrophenylhydrazone (2.4 DNFH) [19] in blood serum, liver, lungs and the myocardium of test animals.

When conducting the research we used the general principles of experiments on animal approved at the National Congress on Bioethics (Kyiv, Ukraine, 2001) and consistent with the provisions of the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes [20]. Statistical analysis of the obtained data we performed using the STATISTICA 6.0 program using parametric Student's t test and the nonparametric Wilcoxon criterion for related samples. Changes were considered as authentic at $p \leq 0.05$ [21].

3. Results and Discussion

The development of the most pathological conditions occurs under free radical mechanism that at the cellular level is characterized by increased production of free radicals, among which a special place belongs to reactive oxygen and nitrogen species (ROS / AFA) [22–23]. Excessive and uncontrolled ROS formation is a trigger in the development of deep oxidative damage of cellular compartments, deepening the pathological process [24]

We studied the content of ROS in neutrophils and lymphocytes in rats' blood of all age groups poisoned by sodium nitrite within 24 hours and 72 hours on the background of 15 day tobacco smoke intoxication (Table 1 and 2).

Table 1: The content of reactive oxygen species (%) in neuthrophils of rats' blood of different age affected with sodium nitrite, on the background of 15 day intoxication with tobacco smoke (M±m; n=72)

Term study, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
intact rats	15,06±0,71	18,47±0,22	19,87±0,86
15 day defeat tobacco smoke	17,19±0,83	28,58±2,53*	25,38±1,95
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	37,84±1,09*	29,05±0,53*	36,64±0,77*
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	40,92±0,58*	35,12±0,72*	42,14±0,60*

Note: * – significant changes between intact rats and rats affected with toxicants ($p \leq 0.05$)

The content of ROS in neutrophils of immature rats after their affection with both toxicants experienced the most pronounced changes. Intoxication by tobacco smoke for 15 days did not lead to a possible increase in ROS content in the

blood. After the introduction of additional toxic factor in rats' body (sodium nitrite) for 24 hours before termination of intoxication by tobacco smoke we observed significantly ($r \leq 0.05$) raising the studied parameters in immature rats in 2.5 times in mature – in 1.6 times and in senile – in 1.8 times compared to the group of control intact. After 72 hours after hitting sodium nitrite in the body affected by tobacco smoke rats we noted more pronounced ROS production by neutrophils of blood which in immature rats exceeded 2.7 times the rate in mature – 1.9 times and in senile – 2.1 times. The most sensitive to toxicants were immature rats, in which hyperproduction of ROS was at the highest level.

In rats' blood lymphocytes we observed similar changes in the content of ROS AFC after their poisoning with sodium nitrite on the background of intoxication with tobacco smoke (Table 2).

Table 2: The content of reactive oxygen species (%) in lymphocytes of rats' blood of different age affected with sodium nitrite, on the background of 15 day intoxication with tobacco smoke (M±m; n=72)

Term study, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
intact rats	9,16±0,24	8,48±0,23	12,18±0,20
15 day defeat tobacco smoke	18,97±1,77*	15,41±1,59*	24,77±1,79*
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	20,30±0,50*	17,07±0,57*	35,82±0,86*
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	25,46±0,25*	18,18±0,58*	38,42±1,63*

Note: * – significant changes between intact rats and rats affected with toxicants ($p \leq 0.05$)

Tobacco smoke showed marked toxic effect on the blood lymphocytes of rats. After the affection by this toxicant we marked active hyperproduction of ROS by blood lymphocytes of rats of all age groups (increase of this indicator was within 1.8–2.0 times). Intake of sodium nitrite in the body of intoxicated rats resulted in a further increase in blood cells of ROS. The highest content of them was observed in senile rats in the last period of the experiment (72 hours after the application of sodium nitrite and day 15 of poisoning by tobacco smoke). During this period the studied parameter was increased in animals of senile age in 3.15 times, in immature – in 2.8 times, in mature – 2.1 times.

It is known that any stress reaction of the body in norm is accompanied by a brief increase in the number of anatomic physiological features (APF) [25–26]. It is caused by a reaction of adaptation to extreme conditions under which the APF act as secondary messenger, participating in the transmitting signal transduction, the expression of a number of genes (proliferation, differentiation, etc.). It is believed that lipid peroxidation may be a key position in signal transduction processes that determine the possibility of cell survival or its death in stressful situations [27]. In marked oxidative stress concentration of formed APF can be increased several times. Under these conditions, toxicity of APF begins to show, accompanied by a strengthening of the processes of oxidative degradation of lipids, proteins, nucleic acids, carbohydrates, display of genotoxic effects, activation of number of protooncogenes [28]. In marked and prolonged oxidative stress the level of APF is increased sharply (in several times), the speed of lipid peroxidation is enlarged; oxidative degradation of proteins, nucleic acids, carbohydrates is intensified. Manifestation of the toxic effect of free radical product leads to structural and metabolic abnormalities in cells with subsequent necrosis [26].

After intoxication by tobacco smoke in blood serum, liver, lungs and the myocardium of rats the content of lipid peroxidation products increases (TBA-AP). Poisoning by toxic smoke of rats with sodium nitrite leads to a more pronounced increase in the content of TBA-AP in the studied tissues (Table 3).

The most pronounced changes the content of TBA-AP experienced in blood serum of mature rats after 15 days of poisoning by tobacco smoke and increased in 1.9 times in blood serum of immature rats in 1.3 times, in senile rats – in 1.6 times. After insertion of sodium nitrite in the body of affected by smoke animals in 72 hours the content of TBA-AP increased in 3 times in mature rats, in 2.1 times – in immature and senile.

In the liver of rats of all age groups after intoxication by tobacco smoke the content of TBA-AP increased in 1.2–1.6 times. Complicated by sodium nitrite poisoning of rats caused a significant ($P \leq 0.05$) increase in this indicator in the liver of rats that is 3.3 times higher than the rate in all age categories.

Poisoning of rats by tobacco smoke resulted in increase of TBA-AP in the lungs of rats, which was the most pronounced in senile animals (intact control level was bigger in 1.9 times).

In rats affected with both toxicants at the end of the experiment (after 72 hours of sodium nitrite poisoning on the background of 15 day poisoning by tobacco smoke) the content of lipid peroxidation products in the lungs of immature animals increased in 1.9 times, in mature – in 2.2 times and in senile – 2.4 times.

A similar trend to increasing of lipid peroxidation products is marked in the myocardium of animals of all groups after intoxication by tobacco smoke. Immature rats were the most sensitive, the contents of TBA-AP in the myocardium of which increased in 1.9 times compared with the norm. This may be due to increased sensitivity to stress of this group of animals. One of the reasons for this situation is the intensified release of catecholamines into the blood in response to stress factors (smoke), including adrenaline, which in high doses showes toxic effect on the myocardium, the more activated the progress of lipid peroxidation and increased content of its intermediate products in the heart. Joining the toxic effects of smoke on the body of another toxicant – sodium nitrite – caused a marked intensification of lipid peroxidation process. The content of TBA-AP in the myocardium of immature and senile rats increased in 2.4 and 2.3 times respectively. Mature rats were the most resistant to both toxicants – in the myocardium of these animals this figure increased in 1.4 times.

Under conditions of oxidative stress the object of damaging actions of excess of free radicals can be not only lipids and proteins and amino acids [29].

It is known [30] that the oxidation of proteins under the action of reactive oxygen forms with aldehydo- or keto group is one of adaptive systems and encourages the multicatalytical protease activation that destroy selectively oxidized proteins. When excessive formation of reactive oxygen, particularly in oxidative stress, protein modification completes the formation

of acid groups of proteins, indicating a deep imbalance of pro- and antioxidant system.

It is believed that oxidative protein modification plays a key role in the molecular mechanisms of oxidative stress and is the trigger to oxidative degradation of other molecules such as lipids and nucleic acids [29].

During the study of oxidative modification of proteins in rats we found the increasing of content of 2.4-dinitrophenylhydrazine as the main (Figure 1) and neutral (Figure 2) in the blood serum of animals of all age groups after the affection of them by sodium nitrite intoxication on the background of tobacco smoke poisoning.

Table 3: The content of TBA-active products in blood serum (mmol / l) and bodies (mmol / kg) in rats of different ages affected by sodium nitrite, against the background of 15 day toxicity by tobacco smoke (M ± m; n = 72)

Term study, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
	blood serum		
intact rats	3,28±0,23	1,85±0,14	2,35±0,14
15 day defeat tobacco smoke	4,28±0,31	3,47±0,29*	3,71±0,28*
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	6,35±0,25*	3,85±0,31*	3,78±0,32*
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	7,06±0,21*	5,50±0,74*	4,92±0,49*
	Liver		
intact rats	15,49±1,28	14,42±0,71	16,55±0,98
15 day defeat tobacco smoke	18,69±1,28	23,50±1,35*	25,12±2,09*
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	28,84±2,34*	40,59±2,94*	47,00±2,95*
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	51,27±4,68*	47,54±1,92*	55,55±2,13*
	Lungs		
intact rats	18,66±0,60	21,82±1,51	21,36±2,13
15 day defeat tobacco smoke	21,47±0,79	34,18±2,29*	39,53±1,97*
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	28,85±1,85*	37,39±2,57*	50,21±1,58*
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	34,72±2,80*	48,61±1,53*	51,81±1,74*
	Myocardium		
intact rats	9,72±0,65	13,35±0,98	13,35±1,28
15 day defeat tobacco smoke	18,16±1,58*	14,95±0,67	17,09±1,58
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	20,08±1,45*	18,16±1,58	21,90±2,10*
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	22,97±1,74*	19,23±1,65*	30,98±3,17*

Note: * – significant changes between intact rats and rats affected with toxicants ($p \leq 0.05$)

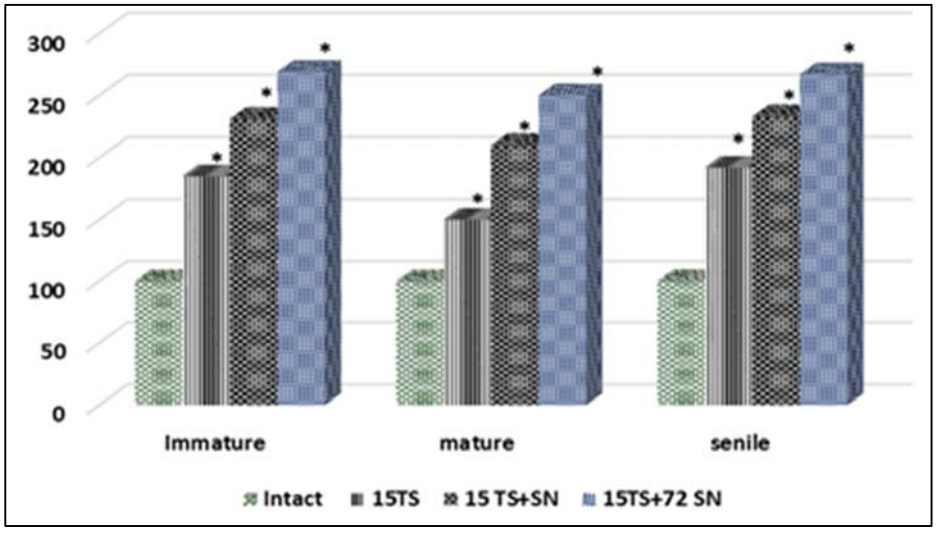

Fig 1

Figure 1 The content 2.4 dinitrophenylhydrazone of the main character (%) in the blood serum of rats affected with sodium nitrite on the background of 15 day intoxication by tobacco smoke

Immature and senile rats were more sensitive to toxic factors than mature animals. The content of 2.4-DNFH of the main character after poisoning by tobacco smoke in the blood serum increased to 185% and 192%, respectively, though in mature rats – up to 150%. When insertion of sodium nitrite into the body of affected by smoke rats the content of this indicator in 72 hours thereafter increased to 269% in immature rats, to 267% in senile and to 250% in mature animals.

A similar increase after the defeat by toxicants we noted in the blood serum 2.4-DNFH neutral character (Figure 2).

Regarding the content of this indicator, the largest increase it sustained in mature animals. After 72 hours of sodium nitrite poisoning after a 15 day toxicity by tobacco smoke in blood serum of mature animals this figure reached 290% in comparing with the intact control level. During this period, the contents of products of neutral oxidative protein modification in serum of immature rats increased by 111% in senile – by 140%.

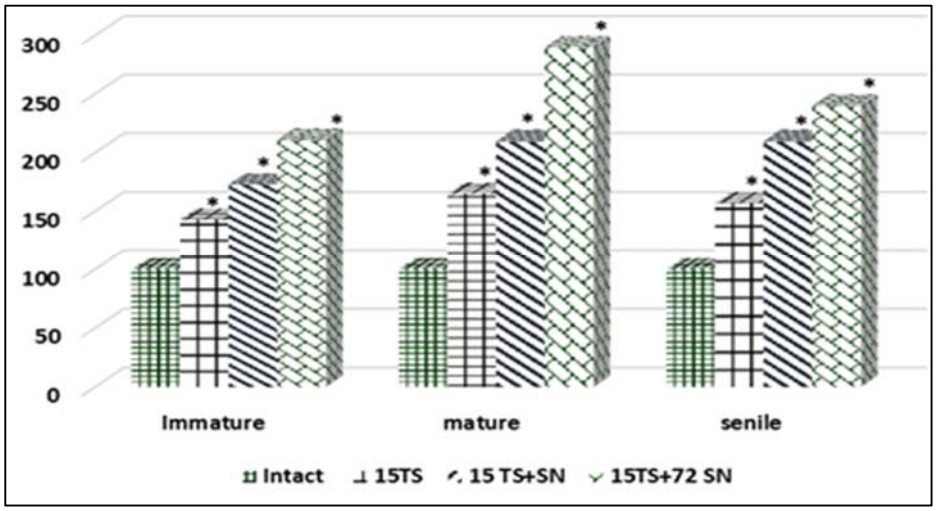

Fig 2

Figure 2 The content of 2.4 dinitrophenylhydrazone of neutral character (%) in the blood serum of rats affected by sodium nitrite on the background of 15 day toxicity by tobacco smoke

Defeat of rats of different ages by tobacco smoke caused the destruction in the biomacromolecules in organs of the studied animals. We noted the increased content of oxidative protein modification products of both factions in the liver, lungs and the myocardium of rats (Table 4).

Table 4: The content of 2.4 dinitrophenylhydrazone in organs (mmol / g of protein) in rats of different ages affected by sodium nitrite, against the background of 15 day toxicity by tobacco smoke (M ± m; n = 72)

Term study, days	2.4-dinitrofenilhidrazone neutral (370 nm)			2.4-dinitrofenilhidrazone primary (430 nm)		
	Groups of experimental animals			Groups of experimental animals		
	immature rats	mature rats	senile rats	immature rats	mature rats	senile rats
	liver					
intact rats	0,101± 0,003	0,072± 0,001	0,083± 0,002	0,042± 0,001	0,026± 0,001	0,041± 0,001
15 day defeat tobacco smoke	0,134± 0,013	0,096± 0,003*	0,107± 0,009	0,047± 0,002	0,029± 0,002	0,047± 0,004
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	0,155± 0,004*	0,108± 0,002*	0,153± 0,003*	0,058± 0,001*	0,037± 0,002*	0,054± 0,003*
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	0,172± 0,002*	0,124± 0,010*	0,156± 0,002*	0,079± 0,002*	0,052± 0,003*	0,075± 0,003*
	lungs					
intact rats	0,039± 0,001	0,028± 0,001	0,035± 0,001	0,030± 0,001	0,019± 0,001	0,027± 0,002
15 day defeat tobacco smoke	0,050± 0,005	0,034± 0,002*	0,050± 0,005	0,032± 0,003	0,022± 0,002	0,029± 0,002
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	0,068± 0,002*	0,042± 0,001*	0,060± 0,002*	0,044± 0,003*	0,026± 0,002	0,034± 0,002
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	0,082± 0,001*	0,056± 0,001*	0,078± 0,002*	0,050± 0,003*	0,033± 0,002*	0,038± 0,003*
	myocardium					
intact rats	0,074± 0,001	0,057± 0,001	0,068± 0,001	0,028± 0,002	0,020± 0,002	0,025± 0,001
15 day defeat tobacco smoke	0,088± 0,007	0,084± 0,002*	0,098± 0,004*	0,032± 0,002	0,024± 0,002	0,030± 0,002
15 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	0,104± 0,002*	0,098± 0,002*	0,110± 0,002*	0,034± 0,002	0,027± 0,001*	0,029± 0,002
15 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	0,108± 0,007*	0,117± 0,006*	0,134± 0,002*	0,044± 0,001*	0,032± 0,002*	0,030± 0,002

Note: This table * – significant changes between intact rats and rats affected with toxicants ($p \leq 0.05$)

After insertion into the body of affected by smoke rats sodium nitrite the content increase of 2.4-DNFH in rats suffered significant alteration ($p \leq 0.05$). After 72 hours of application of toxicant the content of neutral 2.4-DNFH increased in the liver of immature and mature rats in 1.7 times, in senile – 1.9 times. The content of the main character 2.4-DNFH in the liver of immature animals increased in 1.4 times, mature – in 2 times, and senile – in 1.8 times.

The most pronounced increase of oxidative protein modification products content was noted in the lungs of rats of all age groups after the affection by both toxicants (15 days of poisoning by tobacco smoke and 72 hours after application

on the its background sodium nitrite). The content of neutral 2.4-DNFH in this period increased in the lungs of rats in 2.0–2. 2 times depending on the age group, main character 2.4-DNFH – in 1.4–1.7 times.

In the myocardium after the affection by toxicants the content of both oxidative protein modification fractions also was increased and reached its highest level in the last term of the experiment.

Thus, we noted that after the defeat of rats by sodium nitrite on the background of tobacco intoxication, modifying processes of protein molecules that lead to the accumulation of serum and organs of animals and aldehyde- and ketolike of these compounds may occur.

4. Conclusions

Affection of rats by sodium nitrite on the background of 15 day tobacco intoxication leads to increased reactive oxygen content in the blood of rats of all ages that are triggers in activation of free radical processes. 72 hour sodium nitrite poisoning of affected by tobacco smoke rats caused the increase of TBA-active products and 2.4-dinitrophenylhidrazone in blood serum, liver, lungs and heart of rats, suggesting an intensification of the processes of lipid peroxidation and oxidative modification of proteins after poisoning. All above mentioned leads to the development of oxidative stress in animals after defeat, which is more pronounced in immature and senile rats.

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