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Original Research

Combined effects of κ-carrageenan and monosodium glutamate food additives: effect on free radical oxidation

Petro Buchko, Inna Krynytska, Mariya Marushchak*

Department of Functional and Laboratory Diagnostics, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

*Correspondence to: Mariya Marushchak, Department of Functional and Laboratory Diagnostics, I. Horbachevsky Ternopil National Medical University, Majdan Voli, 1., Ternopil, Ukraine 46001, E-mail id: marushchak@tdmu.edu.ua, Phone: +380979981202

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Abstract

Background and aims: The universal mechanism that plays a key role in the implementation of the action of most toxic agents is the activation of free radical processes and the development of oxidative stress. Therefore, the aim of our study was to evaluate the indicators of lipid peroxidation and antioxidant defense system in rat tissues using a solution of κ-carrageenan, monosodium glutamate (MSG), and their combined effects to establish possible relationships. **Materials and methods:** The study was performed on 48 white non-linear male rats that were divided into 4 groups: 1st – control (intact animals); 2nd – animals that were intragastrically injected with κ-carrageenan at a dose of 40 mg/kg for 1 month; 3rd – animals that were intragastrically injected with MSG at a dose of 50 mg/kg for 1 month; 4th – animals that were intragastrically injected with κ-carrageenan and MSG in the above doses. We tested the levels of diene conjugates (DC), active products of thiobarbituric acid (TBA), superoxide dismutase (SOD) and catalase activities (CAT). **Results:** The highest activation of free radical oxidation was observed in lung tissues with the use of MSG and the combined effect of κ-carrageenan and MSG, whereas the lowest studied indicators of lipid peroxidation were registered in all tissues of group 1. The lowest values of the antioxidant defense system parameters were found in 3-d group, while the use of κ-carrageenan caused an increase of SOD and CAT activity with the highest values in liver tissues **Conclusions**: The combined use of a solution of κ-carrageenan and MSG has a negative effect on the processes of free radical oxidation, which is manifested by a statistically significant increase of lipid peroxidation products levels in blood serum, lungs and liver and decreased activity of antioxidant system enzymes in the lungs and liver and in the process significant medium-strength correlations between pro- and antioxidants were established.

Keywords: antioxidant defense system, combined use, κ-carrageenan, lipid peroxidation, monosodium glutamate

Background and Aims

Food additives are widely used in the food industry to maintain food quality, achieve homogeneity, enhance flavor or improve the texture of food [1]. The second place in terms of consumption of additives after the food industry is occupied by the cosmetic industry for the manufacture of lotions, creams, shampoos [2]. Food additives are also used in the textile industry, in biotechnological production for cell immobilization and as a substitute for bacteriological agar [3]. With the expansion of the food additives production, the range of food and industrial products obtained without their use is constantly decreasing [4, 5].

In recent decades, carrageenans have become one of the most popular hydrocolloids

in the food industry [6]. It should be noted that currently the carrageenans have the highest commercial total production (about 60 thousand tons per year), which is 626 million US dollars in annual profit [7]. Their main purpose is the formation of gels, so carrageenans are widely used as emulsifiers, stabilizers, thickeners [8]. Given that carrageenans belong to soluble dietary fibers, they are involved in the regulation of homeostasis, have immunostimulatory and anticoagulant properties, which makes them interesting for use in functional foods [9].

Monosodium glutamate (MSG, monosodium salt of glutamic acid, E621) is used in most food technologies as a food additive and the amount of its use is not controlled. In Ukraine, only in 2000, after the resolution of the Cabinet of Ministers №342 of February 17, MSG was included in the list of permitted food additives in Ukraine [10]. At the same time, the safe daily dose of food additives is ambiguous. Nowadays, there are no reliable data that would show in what doses and under what conditions MSG, consumed constantly in the form of additive E621 is harmful to health [11, 12]. Literature data on the toxic effects of MSG are few and more related to the development of MSG-induced obesity [13–16] and neurotoxicity [17, 18].

Although the safety of carrageenan in food has been debated for decades, E407 retains the status of a safe additive "Generally Regarded as Safe". The Joint FAO/WHO Expert Committee on Food Additives has set its permissible daily dose – up to 75 mg per 1 kg of body weight [19]. In 2015, the Joint Expert Committee on Food Additives of the WHO concluded on the safety of carrageenan in infant formulas [20]. However, there are conflicting data on the safety of carrageenan. A number of studies have shown that carrageenan activates inflammatory signaling pathways, which leads to the induction of pro-inflammatory cytokines [21, 22]. Other studies show that carrageenans pass through the intestinal epithelium; are transferred through a portal blood flow to a liver; bind to TLR4 and induce pro-inflammatory reactions, as well as suppress insulin sensitivity [23].

The universal mechanism that plays a key role in the implementation of the action of most toxic agents is the activation of free radical processes and the development of oxidative stress [24]. However, data on free radical oxidation in body tissues with the consumption of MSG, κ-carrageenan and their combined effects are extremely scarce, so there is a need to study this problem in detail.

Therefore, the aim of our study was to evaluate the indicators of lipid peroxidation and antioxidant defense system in rat tissues using a solution of κ-carrageenan, MSG, and their combined effects to establish possible relationships.

Materials and Methods

The study was performed on 48 white non-linear male rats that were kept on the standard vivarium diet of I.Horbachevsky Ternopil National Medical University. During the work the principles of the European Convention for the Protection of Laboratory Animals were followed. Experimental rats were divided into 4 groups: group 1 – control (intact animals); group 2 – animals that were intragastrically injected with κ-carrageenan at a dose of 40 mg/kg, dissolved in 0.5 mL of distilled water at room temperature for 1 month [25, 26]; group 3 – animals that were intragastrically injected with MSG at a dose of 50 mg/kg, dissolved in 0.5 mL of distilled water at room temperature for 1 month [27]; group 4 – animals that were intragastrically injected with κ-carrageenan and MSG in the above doses.

To prepare 10% of the homogenate, the lung and liver samples, that were taken immediately after euthanasia, were cooled in physiological saline to 1–3°C, dried with filter paper, then scissored and homogenized in 0.05 M Tris-HCl buffer (pH 7.4) using a magnetic homogenizer SilentCrusher S, (Heidolp, Germany) in a ratio of 1:9 (tissue weight: buffer volume). The resulting homogenate was centrifuged for 30 minutes at 3000 rpm in a centrifuge with cooling Hermle Z 32 HK. The supernatant was used for research [28].

For the study in erythrocyte hemolysates, whole blood was centrifuged at 3000 rpm for 15 minutes, the serum was collected, and erythrocytes were washed twice with isotonic sodium chloride solution. The method of Nishikimi N.

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and co-authors was used to prepare hemolysate of erythrocytes [29]. According to the method, to 0.1 mL of washed erythrocytes were added 0.9 mL of Tris-HCl buffer 0.05 M (pH 7.4), 0.25 mL of ethyl alcohol, 0.15 mL of chloroform and the resulting mixture was stirred, centrifuged in a centrifuge for 15 minutes at 3000 rpm with cooling Hermle Z 32 HK. The supernatant was used for research.

The content of diene conjugates (DC) in blood serum (tissue homogenates) was evaluated by the classical method of Z. Placer (1968) in the modification of V.B. Havrylov, M.I. Mishkorudnaya (1983) [30]. To 0.2 mL of serum was added 2.0 mL of isopropanol-heptane mixture (in a ratio of 1: 1). The resulting mixture was shaken for 1 hour, then was added 0.5 mL of hydrochloric acid at pH 2.0, shaken for 2 minutes, then was added 1.0 mL of heptane and continued to shake for 15 minutes. After about 1 hour, photometry of the upper phase was performed at 232 nm against the control sample. The calculation was performed using the molar extinction coefficient of 2.20×10^5 $M^{-1}cm^{-1}$ and expressed in μmol/L of blood serum or μmol/kg of tissue.

The content of active products of thiobarbituric acid (TBA) in the serum was evaluated by the method of M. Uchiyama and M. Mihara [31]. To do this, 0.2 mL of serum was mixed with 2.0 mL of 1.4% solution of orthophosphoric acid and 1.0 mL of 0.5% solution of TBA acid. The mixture was incubated in a water bath for 45 minutes, then cooled and then 2.0 mL of n-butanol was added to extraction of the stained complex. The test tubes were shaken and centrifuged for 20 minutes at 4000 rpm. The upper phase photometry was performed at 532–570 nm against the control sample. The calculation was performed using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and expressed in μmol/L of blood serum or μmol/kg of tissue.

To determine the superoxide dismutase (SOD) activity in hemolysates of erythrocytes was used the method of S. Chevari and co-authors [32]. To do this, to 0.2 mL of erythrocyte hemolysate supernatant (supernatant of lung or liver homogenate) were added 1.3 mL of pyrophosphate buffer (pH = 8.3, molar concentration 0.1 mol/L), 1.0 mL of nitro blue tetrazolium solution, 0.3 mL of phenazine methosulfate solution and 2.0 mL of $NADN_2$ solution (molar concentration 0.2 μmol/L). Next, the samples were kept in a dark place for 10 minutes and photometered at a wavelength of 540 nm on a SF-46 in a 1 cm cuvette against samples without $NADN_2$. Samples with 0.2 mL of phosphate buffer were used as controls instead of hemolysate or homogenate. Enzyme activity was calculated by the formula A_{sod} = T/(100% – T)/C (where A_{sod} is SOD activity; T is the percentage of inhibition, C is the protein concentration), and the percentage of inhibition is $T = (E_k - E_d) \times 100/E_k$ (where T is the percentage of inhibition; E_k is the extinction of the control sample; E_d is the extinction of the test sample). The protein content in body tissues and erythrocyte lysate was determined by the Lowry method [33]. The activity of SOD was expressed in conventional units per 1 mg of protein (con. units/mg of protein).

To determine the catalase activity (CAT) in the supernatant of erythrocyte hemolysate and lung and liver homogenate, a spectrophotometric method was used, which is based on the ability of the enzyme to highly efficiently catalyze the decomposition reaction of hydrogen peroxide to water and oxygen [34]. Phosphate buffer, hydrogen peroxide and Na_2EDTA were added to the control and test samples containing the test enzyme solution. The test tubes were left at room temperature for 10 minutes, and the reaction in the control sample was stopped by adding 1.0 mL of 10% sulfuric acid solution. An appropriate volume of sulfuric acid was added to the test sample after finishing of incubation. CAT was determined on a SF-46 spectrophotometer at a wavelength of 250 nm. The blank sample to account for the spontaneous decomposition reaction of hydrogen peroxide differs in that instead of a sample containing catalase, the same volume of phosphate buffer is added to it. The unit of activity was the amount of hydrogen peroxide that was decomposed during incubation per unit time. The CAT was expressed in μmol of hydrogen peroxide for 1 minute per 1 mg of protein.

Statistical processing of the results was performed using the computer program STATISTICA 7.0. The choice of the method of statistical research was based on the correct distribution of the studied features. Due to the

Table 1: Indicators of DCs in body tissues of rats (μmol/L [kg]) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	2.80 (2.58; 3.13)	4.15 (3.90; 4.23)	4.55 (4.25; 4.83)
Group 1	3.45^ (3.28; 3.60)	5.05^ (4.80; 5.23)	5.90^ (5.80; 6.10)
Group 2	4.6^ (4.48; 4.93)	6.15^ (5.88; 6.40)	6.65^ (6.38; 6.88)
Group 3	6.10^ (5.80; 6.50)	7.10^ (6.88;7.23)	8.05^ (7.68; 8.33)
Kruskal-Wallis coefficient, p	H = 9.89; p = 0.02*	H = 8.52; p = 0.04*	H = 15.22; p = 0.002*

Notes: *Statistically significant results; ^The probability of differences relative to control.

incorrect distribution of quantitative characteristics, their descriptive statistics was carried out as reported as medians and interquartile range (IQ, percentile 25 and percentile 75).

Comparative analysis of quantitative indicators in three or more groups was performed using the Kruskal-Wallis test, which was considered statistically significant at its values of $p < 0.05$. Further pairwise comparison of groups was performed using the Mann-Whitney U test, taking into account the Bonferroni correction when assessing the level of statistical significance.

Results

It was found that the level of DC in the blood, lung and liver homogenates of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 1). In the serum of experimental animals, the level of DC of group 1 probably exceeded the control values by 23.21%, accordingly, group 2 – by 64.29% and group 3 – by 117.86%. In the lung homogenate, the level of DC of group 1 probably exceeded the control values by 21.69%, accordingly, group 2 – by 48.19% and group 3 – by 71.08%. In liver tissues, the level of DC of group 1 probably exceeded the control values by 29.67%, accordingly, group 2 – by 46.15% and group 3 – by 76.92% (Table 1).

The level of DC in the tissues of experimental animals apparently differed in the blood serum and lung tissues of rats of the experimental groups 1 and 2, 1 and 3 with the highest value by the combined use of κ-carrageenan and MSG (Table 2).

It was found that the level of active products of TBA in the blood, lung and liver homogenates of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 3). In the blood serum of experimental animals, the level of TBA of group 1 probably exceeded the control values by 33.75%, accordingly, group 2 – by 58.63% and group 3 – by 95.96%. In the lung homogenate, the level of DC of group 1 probably exceeded the control values by 30.23%, accordingly, group 2 – by 104.65% and group 3 – by 148.84%. In liver tissues, the level of DC of group 1 probably exceeded the control values by 19.10%, accordingly, group 2 – by 37.08% and group 3 – by 56.18% (Table 3).

Table 2: Levels of reliability (p) in the multiple comparison of DCs between the studied groups.

	Group 1	Group 2	Group 3
		Serum	
Group 1	—	**0.025***	**0.01***
Group 2	**0.025***	—	0.07
Group 3	**0.01***	0.07	—
		Lungs	
Group 1	—	**0.03***	**0.046***
Group 2	**0.03***	—	0.27
Group 3	**0.046***	0.27	—
		Liver	
Group 1	—	0.37	**0.03***
Group 2	0.37	—	**0.03***
Group 3	**0.03***	**0.03***	—

Note: *Statistically significant results.

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Table 3: Indicator of active products of TBA in body tissues of rats (μmol/L [kg]) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	3.22 (3.08; 3.34)	2.15 (2.08; 2.43)	4.45 (4.10; 4.65)
Group 1	4.30^ (4.10; 4.50)	2.80^ (2.60; 3.10)	5.30^ (5.08; 5.60)
Group 2	5.10^ (4.98; 5.23)	4.40^ (4.18; 4.50)	6.10^ (5.95; 6.40)
Group 3	6.30^ (6.08; 6.60)	5.35^ (5.10; 5.88)	6.95^ (6.78; 7.13)
Kruskal-Wallis coefficient, p	H = 9.19; p = 0.03*	H = 11.62; p = 0.009*	H = 15.27; p = 0.002*

Notes: *Statistically significant results; ^The probability of differences relative to control.

The level of TBA in the tissues of experimental animals apparently differed in the blood serum of rats of all experimental groups, in the lung homogenate in rats of groups 1 and 2, 1 and 3, in the liver tissue in rats of groups 1 and 3, 2 and 3 with the highest value by the combined use of κ-carrageenan and MSG (Table 4).

When comparing the indicators of LPO (relative to control), the highest values of the studied indicators were found in 3 experimental groups, where the highest activation of free radical oxidation was observed in lung tissues with the use of MSG and the combined effect of κ-carrageenan and MSG, whereas the lowest studied indicators of LPO were registered in all tissues of experimental group 1 (Figure 1).

It was found that the level of SOD activity in the homogenate of the lungs and liver of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 5). In the blood serum of experimental animals, the level of SOD activity in all experimental groups did not differ statistically significantly from the control. In lung tissues, SOD activity was significantly higher in animals of group 1 (by 21.77%) and, accordingly, lower in animals of group 2 (by 23.39%) and group 3 (by 56.18%) against control values. In liver tissues, the activity of SOD was significantly higher in animals of group 1 (by 40.74%) and, accordingly,

Table 4: Levels of reliability (p) in the multiple comparison of the active products of TBA between the studied groups.

	Group 1	Group 2	Group 3
		Serum	
Group 1	—	**0.045***	**0.001***
Group 2	**0.045***	—	**0.03***
Group 3	**0.001***	**0.01***	—
		Lungs	
Group 1	—	**0.03***	**0.046***
Group 2	**0.03***	—	0.27
Group 3	**0.046***	0.27	—
		Liver	
Group 1	—	0.37	**0.03***
Group 2	0.37	—	**0.03***
Group 3	**0.03***	**0.03***	—

Note: *Statistically significant results.

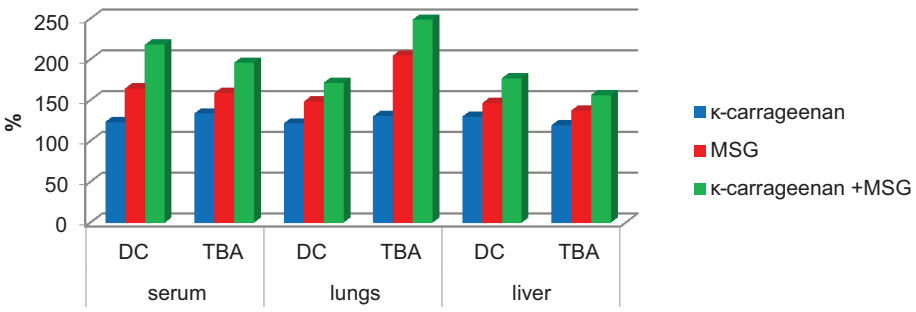

Figure 1: Comparison of lipid peroxidation indicators (%) in the tissues of rats under the combined effects of food additives.

lower in animals of group 2 (by 25.23%) and group 3 (by 59.95%) against control values (Table 5). The activity of SOD in the lungs of rats apparently differed in animals of groups 2 and 3 ($p = 0.01$) and, accordingly, in the liver of rats – in groups 2 and 3 ($p = 0.03$) with the highest values by the combined use of κ-carrageenan and MSG.

It was found that the level of CAT in the homogenate of the lungs and liver of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 6). In the blood serum of experimental animals, the level of CAT in all experimental groups did not differ statistically significantly from the control. In lung tissues, the level of CAT was significantly lower in animals of group 2 (29.26%) and group 3 (45.66%) against control values. In liver tissues, the level of CAT was significantly lower in animals of group 2 (38.25%) and group 3 (43.86%) against control values (Table 6).

The CAT in the lungs of rats apparently differed in animals of groups 1 and 3 ($p = 0.02$) and, accordingly, in the liver of rats – in 1 and 3 ($p = 0.03$) and 2 and 3 groups ($p = 0.03$) with the highest value by the combined use of κ-carrageenan and sodium glutamate.

When comparing the enzyme link indicators of the antioxidant defense system (relative to control), the lowest values of the studied parameters were found in 3-D experimental group, while the use of κ-carrageenan caused an increase of SOD and CAT activity with the highest values in liver tissues (Figure 2).

Correlation analysis showed a direct relationship between the activity of SOD and the concentration of TBA in the blood serum and the studied indicators of LPO in the liver of rats injected with κ-carrageenan. In group 2, a negative relationship was established between the activity of CAT and the studied indicators of LPO in the lungs and liver. It is also worth noting the presence of the moderate inverse relationship between SOD activity and the concentration of TBA in the homogenate of the lungs and liver of rats of group 2. The combined use of κ-carrageenan and MSG revealed a negative relationship between the indicators of LPO and enzymes of the antioxidant system in liver tissues, as well as between the activity of CAT and the studied products of LPO in the lungs (Table 7).

Table 5: Indicator of SOD activity in the tissues of rats (con. units/mg of protein) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	31.55 (30.6; 32.18)	18.60 (18.48; 19.13)	21.60 (21.18; 22.53)
Group 1	34.95 (33.78; 36.33)	22.65^ (21.98; 23.43)	30.40^ (29.38; 31.25)
Group 2	27.05 (26.30; 27.45)	14.25^ (13.50; 15.88)	16.15^ (15.78; 16.45)
Group 3	24.65 (23.55; 25.35)	8.15^ (7.8; 8.50)	8.65^ (8.20; 9.10)
Kruskal-Wallis coefficient, p	H = 3.91; p < 0.05	H = 6.08*; p = 0.047	H = 6.31*; p = 0.043

Notes: *Statistically significant results; ^The probability of differences relative to control.

Table 6: Indicator of CAT in the tissues of rats (con. units/mg of protein) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	17.55 (16.98; 18.10)	15.55 (15.13; 15.88)	14.25 (13.75; 14.83)
Group 1	18.95 (18.48; 19.58)	16.40 (16.18; 16.88)	16.10 (15.90; 16.23)
Group 2	12.20 (11.80; 12.80)	11.00 (10.55; 11.58)	8.80^ (8.45; 9.05)
Group 3	11.40 (10.68; 11.75)	8.45^ (8.20; 8.83)	8.00^ (7.78; 8.20)
Kruskal-Wallis coefficient, p	H = 4.41; p < 0.05	H = 6.31; p = 0.04*	H = 8.03; p = 0.045*

Notes: *Statistically significant results; ^The probability of differences relative to control.

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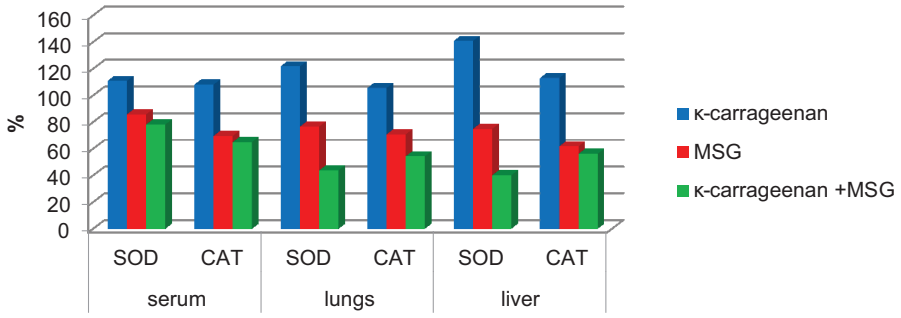

Figure 2: Comparison of indicators of the enzyme link of the antioxidant defense system (%) in the tissues of the body of rats under the combined effects of food additives.

Table 7: Correlation between the indicators of lipid peroxidation and indicators of antioxidant protection of tissues of rats under the combined effects of food additives.

Variables	Group 1		Group 2		Group 3	
	SOD	CAT	SOD	CAT	SOD	CAT
			Serum			
DC	r = 0.38; p = 0.07	r = 0.32; p = 0.13	r = –0.31; p = 0.14	r = –0.25; p = 0.23	r = –0.13; p = 0.52	r = –0.11; p = 0.41
TBA	**r = 0.42*; p = 0.04**	r = 0.40; p = 0.06	r = –0.30; p = 0.15	r = –0.34; p = 0.11	r = –0.27; p = 0.21	r = –0.29; p = 0.17
			Lungs			
DC	r = 0.11; p = 0.41	r = 0.13; p = 0.52	r = –0.38; p = 0.07	**r = –0.46*; p = 0.02**	r = –0.37; p = 0.07	**r = –0.48*; p = 0.02**
TBA	r = 0.15; p = 0.41	r = 0.18; p = 0.52	**r = –0.42*; p = 0.04**	**r = –0.48*; p = 0.02**	r = –0.29; p = 0.17	**r = –0.55*; p = 0.005**
			Liver			
DC	**r = 0.55*; p = 0.005**	r = 0.39; p = 0.06	r = –0.37; p = 0.07	**r = –0.46*; p = 0.02**	**r = –0.42*; p = 0.04**	**r = –0.52*; p = 0.01**
TBA	**r = 0.52*; p = 0.01**	r = 0.37; p = 0.07	**r = –0.55*; p = 0.005**	**r = –0.52*; p = 0.01**	**r = –0.48*; p = 0.02**	**r = –0.46*; p = 0.02**

Note 1: r is the correlation coefficient; p is the level of reliability.
Note 2: *Statistically significant results.

Discussion

Studies over the past 10 years have confirmed that MSG causes disorders such as headache, asthma, diabetes, muscle pain, atrial fibrillation, ischemia, trauma, convulsions, stroke, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, Parkinson's disease, depression, multiple sclerosis, schizophrenia, obsessive-compulsive disorder, epilepsy, drug addiction, attention deficiency/hyperactivity disorder, fronto-temporal dementia and autism [35–37]. The development of these disorders is based on typical pathological processes. The universal mechanism that plays a key role in the implementation of the action of most toxic agents is the activation of free radical processes and the development of oxidative stress [38]. The mechanism of cell damage by free radicals consists of their ability to initiate the peroxidation of lipids and proteins, covalently bind to bio macromolecules, and generate reactive forms of oxygen and nitrogen that are highly toxic and capable of initiating cell death [39, 40].

Our results indicate the activation of free radical oxidation and reduced activity of the

enzymatic link of the antioxidant defense system in the blood, lung tissues and liver of rats under intragastric administration of MSG at a dose of 50 mg/kg, which may be associated with direct hyperproduction of oxygen due to the introduction of the studied food additive. It should be noted that the liver has an autonomous system of detoxification of the body, where normally there is a leakage of unpaired electrons, and hence reactive oxygen species. Respiratory and metabolic functions of the lungs are closely related to lipid metabolism, which makes lung tissues prone to damage by various mechanisms, such as lipid peroxidation, oxidative modification of proteins, and DNA damage. This, in turn, can affect the state of phospholipids in the human body and affect the composition of the lung surfactant, which is the largest biological membrane in the human body, which includes saturated and unsaturated fatty acids, where free radical processes are also take place that regulate surfactant properties and support ventilation of lung tissue [41]. In the work of O. Onyema and co-authors is showed that long-term use of MSG causes oxidative stress and increased free radical oxidation of lipids and proteins in the body [42]. Decreased levels of major antioxidant enzymes and increased lipid peroxidation in the kidneys of rats that were consuming MSG have been demonstrated in works of M.V. Paul and co-authors [43] and M. Thomas and co-authors [44]. The authors note that the depletion of antioxidant protection correlates with an increase of LPO. L.P. Gordienko and co-authors found that under conditions of MSG-induced obesity in the salivary glands of experimental rats was significantly increased the content of TBA active products compared to the control. In the tissues of the salivary glands of animals, catalase and SOD activity was significantly reduced relative to control animals [13]. R.Z. Hamza and co-authors investigated that MSG causes an increase of LPO levels in parallel with a significant decrease of SOD activity and CA in testicular tissues [45]. The results of our study can be explained by the data of Tezcan and co-authors [46], who note that TBA is one of the ending products of LPO, and it is formed as a reaction product of cyclooxygenase in the metabolism of prostaglandins, which confirms the presence of oxidative stress in rats treated with MSG. The study of S. Kumari and co-authors [47] demonstrates that its toxicity is directly related to hyperpolarization of the mitochondrial membrane, increased ROS production, and increased oxygen consumption by mitochondria, mitochondrial dynamic imbalance up to cleavage and activation of autophagy. Regarding changes of SOD and catalase activities under the isolated use of MSG, results are also consistent with the studies of V.V. Bevzo, who showed that a 4-week administration of 3% MSG solution at a rate of 30 mg/kg of body weight of rats reduced the activity of these enzymes in both blood serum and liver homogenate [14]. Another study showed that long-term administration of MSG causes an increase in the generation of ROS by blood neutrophils by 40.3%. Prolonged administration of MSG causes an increase of TBA content by 56.2% in blood serum and 2.1 times in lung homogenate [48, 49]. It should be noted that MSG receptors are present in many tissues of the body, including the brain, heart, lungs, pancreas, where glutamate acts as a neurotransmitter [50]. Consumption of large amounts of MSG can overload MSG receptors in the body and artificially increase the level of free MSG in the body [51].

The results of our study showed a probable increase of lipid peroxidation in parallel with an increase of SOD activity in lung and liver tissues with the use of κ-carrageenan at a dose of 40 mg/kg. Scientific data indicate that under normal digestion, due to acid hydrolysis, κ-carrageenan is broken down into low and high molecular particles that trigger free radical processes in the small intestinal wall at both studied concentrations of κ-carrageenan [52, 53]. The authors note that the use of 1.0% solution of κ-carrageenan is accompanied by the involvement of organs in the pathological process, in particular, the liver and heart, which justifies the generalization of biochemical processes at the body level. Previous studies indicate the involvement of macrophages in the uptake of κ-carrageenan with the formation of heterolysosomes, which leads to the realization of the harmful effects of lysosomal enzymes. On the other hand, macrophages are known to be a source of free radicals [54]. Activation of free radical oxidation processes in liver tissues may be due, according to O.S. Tkachenko and co-authors, as a direct stimulation of the generation of reactive

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oxygen species by κ-carrageenan, and indirectly by the stimulating effect of tumor necrosis factor-alpha, the concentration of which increases with the use of carrageenan [55].

In modern conditions, people consume different food supplements in different combinations and different concentrations every day, which necessitates their combined effect on the body. According to the results of our study, under the combined effect of κ-carrageenan and MSG, lipid peroxidation and depletion of the antioxidant defense system in the lungs and liver increase, with a probable medium-strength relationship between pro- and antioxidants. At present, it is difficult to say what effect the studied food additives have in combination: additive effect or potentiation of effects. On the one hand, both dietary supplements affect oxidative stress; on the other hand, the toxic effects of the studied supplements are also associated with other mechanisms: inflammation under the effect of κ-carrageenan [56] and neurotoxicity under the effect of MSG [57]. However, both with the use of MSG and its combined effect with κ-carrageenan, according to our results, there is a depletion of antioxidant reserves, while with the use of κ-carrageenan, antioxidant activity increases, thereby reducing the negative impact on tissues. Therefore, we consider the dominant negative effect of MSG on the processes of free radical oxidation.

Conclusions

The combined use of a solution of κ-carrageenan and MSG has a negative effect on the processes of free radical oxidation, which is manifested by a statistically significant increase of lipid peroxidation products levels in blood serum, lungs and liver and decreased activity of antioxidant system enzymes in the lungs and liver and in the process significant medium-strength correlations between pro- and antioxidants were established.

Conflict of Interest

The authors declare no conflict of interest.

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