

Influence of *Stachys sieboldii* Miq. root powder on changes in neural system parameters in growing male rats on a high fat and sucrose diet

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Abstract

The aim of this study is to investigate the effects on the nervous system of the use of *Stachys sieboldii* root powder, in growing male rats on a high fat and sucrose (HFHS) diet. The animals were divided into three groups: Intact – normal rodent chow, HFHS – high fat and sucrose diet, HFHS + *Stachys* – high fat and sucrose diet with *Stachys* supplementation. After 30 days of the experiment, the animals were subjected to the "Open Field" test to study the changes in behavioural responses. Biochemical studies of changes in the concentration of lipid peroxidation products in brain homogenates of growing rats were also carried out. Growing males on the HFHS diet exhibit altered nervous system functioning. Animals show apathy, their motor activity and indicators of exploratory behaviour are reduced and the level of emotional reactivity increases. On the part of biochemical changes – the level of all indicators of lipid peroxidation increases significantly. The introduction of *Stachys* root powder reduces the negative effect of the diet on the organism of animals – they significantly increase the indicators of exploratory behaviour, increase locomotor activity and reduce emotional reactivity, as well as there is a decrease in the concentration of all products of free-radical oxidation in the brain homogenate. Our study showed that diets high in fat and sucrose had negative effects on growing male rats. The introduction of *Stachys sieboldii* root powder into the diet improved both behavioural response scores and antioxidant processes in the brain.

Keywords: *Stachys sieboldii*, Open field, Lipid peroxidation, Conjugated dienes, Ketodienes

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Introduction

Deviant behavior in children and teenagers is a very common phenomenon in the modern world. Not only

parents, but also teachers talk about this problem, because deviant behavior negatively affects both life in society and performance in school and other educational institutions (Teymoori et al., 2018).



Moreover, teenagers with significantly deviant behavior (e.g., physical aggression) are more likely to develop antisocial behavior, and this may be associated with health risks (Evans et al., 2019).

Many factors influence deviant behavior in early childhood. One of the most obvious factors is an unbalanced diet, high in fat, sugar, and deficient in vitamins and micronutrients (Wu et al., 2020).

An unbalanced diet may be the cause of attention deficit disorder, aggression, hyperactivity and other similar behavioral disorders in children and teenagers (Qureshi et al., 2021). Studies have shown that dietary components such as saturated fats, sugar, and certain food additives may be responsible for the development of behavioral problems (Lee et al., 2022).

An unbalanced diet can lead to the development of depression in adolescents, through physiological mechanisms such as impaired production of neurotransmitters (e.g., serotonin and tryptophan) (Otsuka et al., 2015). In addition, lipids from our diet affect the function of brain cell membranes. This may be one of the reasons for the development of depression. There have been studies proving that diets low in n-3 polyunsaturated fatty acids may cause behaviors associated with depression and increased anxiety due to the fact that n-3 polyunsaturated fatty acids are involved in the process of cell membrane fluidity or are part of the membranes of brain cells (Müller et al., 2015). There have also been studies showing that diets high in fat and sugar can also reduce levels of brain-derived neurotrophic factor in the hippocampus, which may be another reason for the development of symptoms of depression (Atak et al., 2023). There have been many studies examining the relationship between unbalanced diets and the development of depression, and most studies have found a positive relationship between these factors (Oddy et al., 2018). For example, when studying the effect of a Mediterranean diet (rich in grains, legumes, vegetables, fruits, and fish) on human behavior, a relationship inversely proportional to the risk of depression was found (Shafiei et al., 2019). Consistent with this trend, the so-called "Western" dietary pattern (rich in fats and sugars) and prevalent in Northern Europe and the United States is considered an important risk factor for depression (Saul et al., 2022).

The genus *Stachys* L., a large member of the family Lamiaceae, is distributed throughout Europe, the

Caucasus, Turkey, Iran, Central Asia and China (Tundis et al., 2014). The extract of *Stachys* species contains important bioactive phenolic compounds such as – chlorogenic acid, catechin and rosmarinic acid and has promising antioxidant effects beneficial for health. Thus, these medicinal herbs can be considered as useful ingredients in food and pharmaceutical products (Bahadori et al., 2020). The range of therapeutic properties attributed to these species has been related to their phytochemical composition. The above-ground part of the plant contains betaine bases such as stachydrine, betonicine, turicine, and trigonelline. In addition, there are choline, allantoin, as well as tannins, resins, essential oil, ascorbic acid and organic acids (Gören, 2014).

Stachys tubercles contain a large amount of protein and carbohydrates. The carbohydrates contain a unique compound – stachyose tetrasaccharide, which has an insulin-like effect and effectively reduces blood glucose levels (Slobodianiuk et al., 2021). Stachyose oligosaccharides stimulate the growth of beneficial intestinal microflora. Stachyose is not digested in the intestine, and reaches the large intestine in an unchanged form, where it is fermented only by obligate saccharolytic microflora, the products of which are a favorable factor in the reproduction of lacto- and bifidobacteria (Yin et al., 2006). Biologically active substances are accumulated in *Stachys* nodules: iridoid glycosides, phenolic compounds, ascorbic acid, alkaloids. The presence of ascorbic acid (up to 15%) and free amino acids increase the healing properties of *Stachys*. *Stachys* tubers are dominated by high concentrations of free amino acids such as L-aspartic acid, L-proline and L-phenylalanine. It also contains amino acids such as proline, alanine, isoleucine, leucine and phenylalanine which enhance the antioxidant properties of this plant (Marchyshyn et al., 2022).

Among macronutrients in *Stachys* nodules, potassium, silicon and phosphorus predominate, while micronutrients include iron, zinc, chromium, rubidium, as well as niobium and selenium, which are rarely found in plants (Zakharova et al., 2013). The combination of high concentrations of potassium and boron in *Stachys* tubercles may have a positive effect on carbohydrate metabolism. *Stachys* tuber can serve as a source of chromium for the elderly and diabetic patients, whose bodies poorly digest carbohydrates, and chromium enhances the metabolic processes of these compounds, participating in the



regulation of blood glucose levels (Molchanova et al., 2021). Therefore, the presence in stachys tetrasaccharide stachyose nodules with insulin-like effect of biologically active substances: antioxidants - iridoid glycosides, phenolic compounds, ascorbic acid, selenium and deficient macro- and microelements: potassium, chromium, zinc, organogenic silicon, determines multifunctional pharmacological activity of Stachys nodules. The results of phytochemical analysis of plant raw material of Stachys tubers, conducted at the Institute of Plant Biology and Biotechnology, MES RK, Almaty, showed that the tubers contain the largest number of amino acids, carbohydrates, triterpenoid glycosides, in small amounts - alkaloids, flavonoids, in traces - organic acids, absent - tannins, coumarins and anthraquinones (Sarsenbaev et al., 2012). The phenolic compounds of Stachys include quercetin, chlorogenic acid, luteolin, ferulic acid. These phenolic compounds have been shown to have preventive and therapeutic effects in psychiatric disorders, which means that the powder from the tubers of this plant has promising prospects as an anxiolytic and antidepressant agent (Jahani et al., 2019).

The aim of this study is to investigate the effects on the nervous system of the use of *Stachys sieboldii* root powder, in growing male rats on a high fat and sucrose diet.

Material and Methods

Preparation of the material

The *Stachys sieboldii* root powder used in this experiment was produced at the Phytochemistry Holding, Kazakhstan, Karaganda, in July 2021. The roots were washed three times with tap water to remove sand and dust adhering to the surface. The washed *Stachys sieboldii* roots were lyophilised for 72 h and ground into powder. The *Stachys sieboldii* powder was then stored at -70°C until experimental rodent diets were made. The content, % per absolute dry weight of biologically active compounds in the studied Stachys nodules: the sum of phenolic compounds – 3.86%, simple phenolic compounds and phenolcarboxylic acids – 0.65%, flavonoids – 0.44%, condensed and polymeric phenolic compounds – 2.77% (Gins et al., 2015).

Animal experiments and diets

As an object of the experimental study, we took

immature rats of Wistar line in the number of 30 individuals. The average weight of animals at the beginning of the experiment is 50-70 g. Immature rats were selected for the experiment on the 21st day of life. Experimental animals were kept at a temperature of $18 \pm 2^\circ\text{C}$, humidity $55\% \pm 5\%$ and 12-hour light-dark cycle (8:00 – 20:00) in the vivarium. The animals had free access to food and water during the experimental period. Experimental animals were randomly assigned to one of three experimental groups (n=10 per group).

Group 1 – 10 immature males maintained on an all-vivarium, balanced diet.

Group 2 – 10 immature males fed a daily high fat and sucrose (HFHS) diet.

Group 3 – 10 immature males fed daily with a high fat and sucrose diet supplemented with Stachys (HFHS + Stachys) (Table 1).

Table-1. Composition of the experimental ration

| Components (g/kg) | Group 1 Intact normal rodent chow | Group 2 HFHS | Group 3 HFHS + Stachys |
|--|--|-----------------|------------------------------|
| Corn | 200 | 80 | 80 |
| Rice | 200 | 200 | 200 |
| Bone meal | 120 | 120 | 120 |
| Sucrose | - | 100 | 100 |
| Soy oil | 75 | - | - |
| Lard | – | 200 | 200 |
| Gluten | 200 | 200 | 200 |
| Salt | 3.5 | 3.5 | 3.5 |
| Mineral mix | 35 | 35 | 35 |
| Vitamin mix | 16.5 | 16.5 | 16.5 |
| Inert material | 150 | 45 | 45 |
| Total (g) | 1000 | 1000 | 1000 |
| Nutrient composition (%) | | | |
| Protein | 24.8 | 19.2 | 19.2 |
| Carbohydrate | 49.6 | 43.4 | 43.4 |
| Lipids | 25.6 | 37.4 | 37.4 |
| <i>Stachys sieboldii</i> Miq. root powder | 0,0 | 0,0 | 50 |
| Energy density (kcal/g) | 3.55 | 4.49 | 4.49 |

The period of the experiment was 30 days.



The study was conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes, the requirements of GLP OECD, the Rules of Good Laboratory Practice of the EAEC #81, the order of the MHSD RK #392 of 25 May 2015. The study was approved by the decision of the Bioethics Committee of "Medical University of Karaganda" on 17.06.2021, protocol № 165.

Determination of physiological parameters

To assess the change in behavioural patterns, the «Open field» test was performed on day 30 of the study. The "Open field" was a circular area of 150 cm diameter, the entire area of which was divided into 16 squares and bounded by opaque sides, 50 cm high. The operator held the base of the rat's tail and placed the rat in the middle of the box, after which motor activity was monitored visually for 2 minutes. Horizontal locomotor activity (number of squares crossed), vertical activity, number of acts of grooming, defecation and urination were recorded. After 2 minutes the rats were removed from the open field. After each rat, the floor of the platform was thoroughly wiped with a towel moistened with a weak alcohol solution (Li et al., 2017).

Horizontal locomotor activity of animals in the "Open field" includes running along different trajectories, up to circling around one place. The main criterion for identifying this form of behaviour is the participation of all four legs in the animal's movement. The floor of the arena is divided into three rows of sectors of equal area, so it is convenient to take one crossed sector as a unit of movement for visual registration of behaviour. Movement was recorded as follows: if the animal was within one sector (with all four paws) and then moved to the adjacent sector (hind paws crossed the line separating them), it was considered that one sector was crossed.

The vertical motor activity of animals in the "Open field" was represented by two types of stands: Climbing – when the hind paws of the animal remained on the floor of the platform, and the front paws rested on the border of the platform and Rearing – when the hind paws of the animal remained on the floor of the platform, and the front paws remained on the weight. The results were calculated on the basis of total vertical activity, without dividing it into Climbing and Rearing. The results were calculated on the basis of total vertical

activity, without dividing it into Climbing and Rearing. Animal grooming in the "Open field" is by washing the eye area, putting the paws behind the ears and moving on to washing the whole head, paws, sides, torso, ano-genital area, tail. To determine the level of defecation, we counted the number of boluses (Yepifantseva et al., 2020).

Biochemical research methods

Euthanasia and brain tissue harvesting

All rats were killed by complete decapitation with sharp scissors. The brain of all rats was immediately removed.

Biochemical assays in brain homogenates

For biochemical analysis, fresh brain tissue was washed with chilled 10% saline solution. Tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10000 rpm for 15 min at 4°C. The resulting supernatants were separated, frozen in liquid nitrogen and then stored at -80°C until further processing.

Determination of conjugated dienes, ketodienes and Schiff bases

0.3 ml of supernatant was diluted with phosphate buffer (pH 7.4) at a ratio of 1:33 and 1 ml was placed in a tube with a fitted stopper, mixed with 9 ml of heptane-isopropyl alcohol mixture (1:1 by volume), shaken for 1 min. Then centrifuged for 5 min at 3000 rpm and incubated for 1 min for phase separation. 3 ml of the upper heptane layer was taken into another tube and the optical density was determined at 232 nm (for Conjugated Dienes) and 268 nm (for Ketodienes). The ratio of wavelengths E400 /E220 allows to judge the level of Schiff bases (Ushkalova and Kadochnikova, 1987).

Measurement of MDA (Malondialdehyde)

For examination 0.3 ml of supernatant was taken, to which 2.4 ml of 1/12 n. H₂SO₄ and 0.3 ml of 10% phosphorus-tungstic acid were added and mixed thoroughly. After 10 min centrifugation at 3000 rpm for 10 min, the resulting precipitate was washed. 10 min, the resulting precipitate was washed twice with 1 ml of water. The washed precipitate was dissolved in 3 ml of water, 1 ml of freshly prepared aqueous solution of TBC (thiobarbituric acid) in acetic acid was added (80g of TBC dissolved at warmed temperature). TBC was dissolved on heating in 5 ml of H₂O and 5 ml of glacial acetic acid). The colour reaction was carried out at 96°C. The reaction was



stopped after 60 min by cooling the samples in cold water. Samples were centrifuged for 15 min at 3000 rpm to remove turbidity before examination in a spectrophotometer. Optical density was measured at 532 nm in 10 mm cuvettes. The control was distilled water. The amount of MDA was calculated according to the formula $C=E/\xi$, where E – optical density of the sample, ξ – molar extinction coefficient equal to $1.56 \cdot 10^{-5}$, C – MDA concentration expressed in $\mu\text{mols per 1 ml of supernatant}$ (Draper and Hadley, 1990).

Spectrophotometric analysis was performed on a UV-VIS PD-303UV digital spectrophotometer manufactured by APEL, Japan.

Statistical analysis

All data were analysed using independent T-test using Graph Pad Prism 8. Data are presented as mean \pm standard error. A P value <0.05 was considered a significant difference. Correlation between behavioural parameters and biochemical data was analysed using Pearson's correlation test.

Results

The following data were obtained as a result of the experiment. In growing males in the HFHS group, compared to those in the intact group, vertical activity levels decreased 3.28-fold ($p<0.0001$), horizontal activity scores decreased 2.3-fold ($p<0.0001$), the number of grooming acts increased 2.2-fold ($p=0.1374$), and urination scores decreased 20% ($p=0.7541$). The number of defecation acts increased by 60% (Figure 1).

Intact – normal rodent chow; HFHS – high fat and sucrose diet; HFHS + Stachys – high fat and sucrose diet with Stachys supplementation

Behavioural responses of growing males in the HFHS + Stachys group changed as follows: vertical activity increased 2.5-fold compared to the HFHS group ($p=0.0002$) and was 30% lower than the intact group. Horizontal activity scores increased 40% compared to the HFHS group ($p<0.0001$), but remained 50% lower than the intact group. The number of grooming acts increased slightly compared to that of the HFHS group ($p=0.0723$), and these rates were 2-fold higher than those of the intact group. The rate of urination acts increased by 10% compared to the HFHS group ($p=0.5258$). The number of acts of defecation decreased 2-fold compared to the HFHS group ($p=0.2237$), and were 20% lower than the intact group (Figure 1).

Comparison of indices of PO level in brain homogenate of growing rats gave the following data: in the HFHS group, compared to the indicators of the intact group of growing rats, the level of Conjugated dienes increased 2.6 times ($p<0.0001$), the level of ketodienes increased 3.6 times ($p<0.0001$), the indicators of malonic dialdehyde increased 2 times ($p<0.0001$), and the level of Schiff bases increased 15 times ($p<0.0001$) (Figure 2).

Pearson correlation between behavioural patterns showed that vertical activity is positively correlated with horizontal activity (high correlation) and urination (medium correlation) and negatively correlated with grooming (medium correlation) and defecation (high correlation).



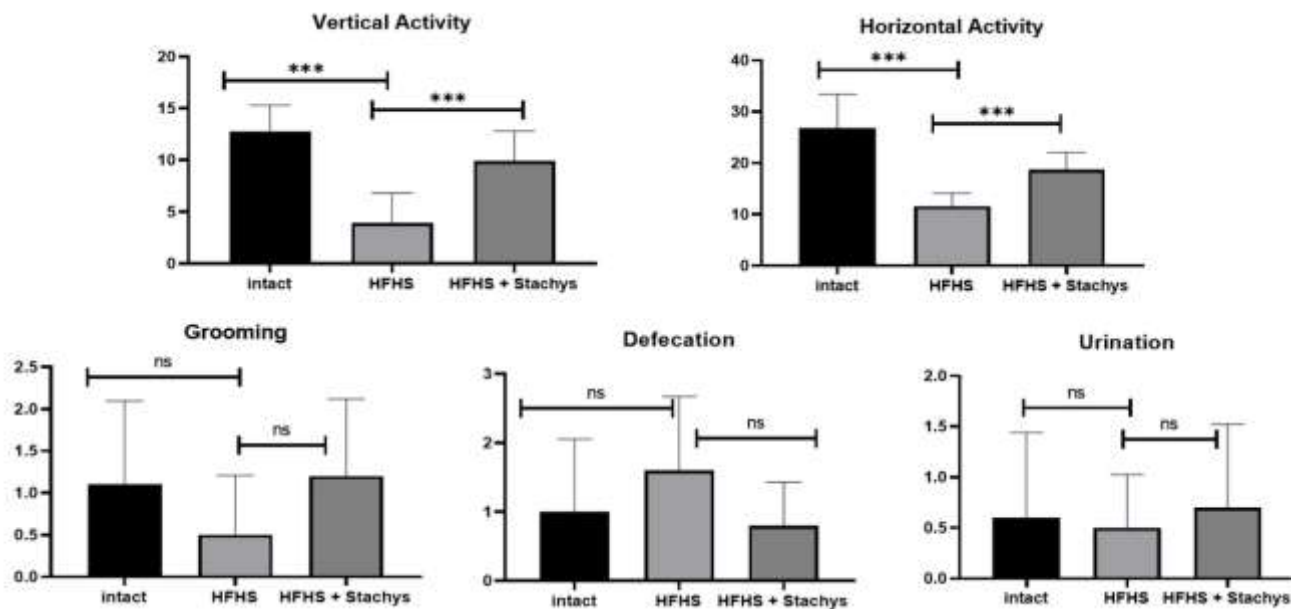


Figure-1. Changes in behavioural response parameters in growing male rats, in the three groups studied

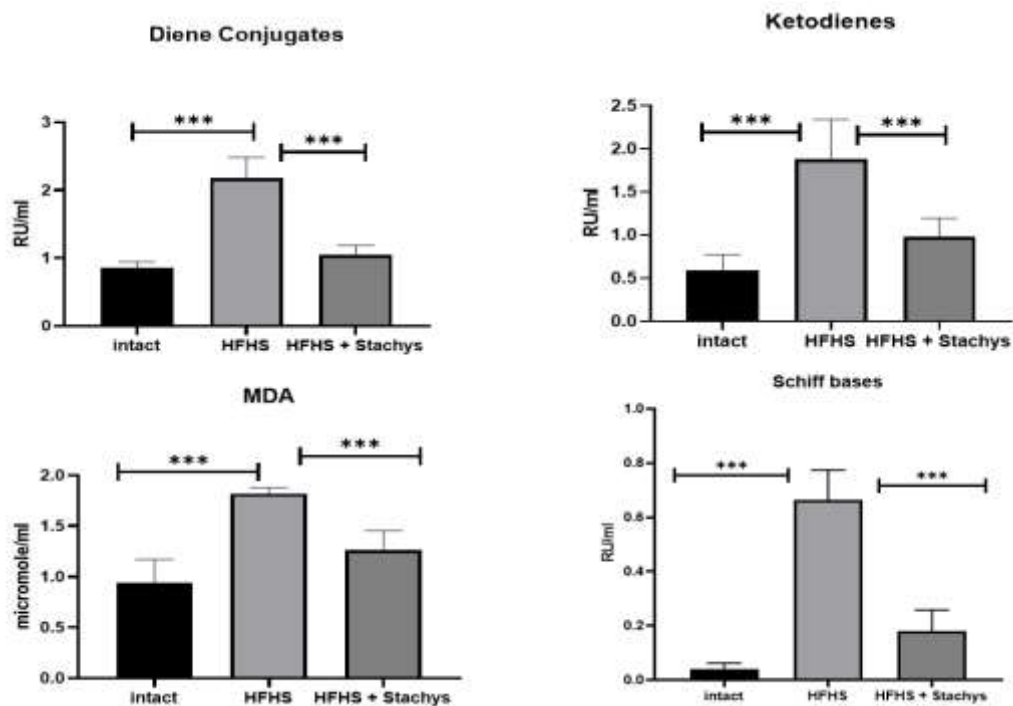


Figure-2. Changes in lipid peroxidation products in growing male rats, in the three groups studied

Table-2. Pearson correlation between behavioural parameters in the "Open Field" test and indicators of lipid peroxidation products

| | VA | HA | Groom | Urination | Defecation | MDA | DK | KD | SHO |
|------------|---------|---------|---------|-----------|------------|---------|---------|---------|---------|
| VA | 1 | 0.9721 | -0.6565 | 0.6609 | -0.8431 | -0.9316 | -0.9818 | -0.9995 | -0.9940 |
| HA | 0.9721 | 1 | -0.8149 | 0.4667 | -0.6937 | -0.9908 | -0.9101 | -0.9644 | -0.9408 |
| Groom. | -0.6565 | -0.8149 | 1 | 0.1320 | 0.14803 | 0.8858 | 0.5017 | 0.6329 | 0.5703 |
| Urination | 0.6609 | 0.4667 | 0.1320 | 1 | -0.9607 | -0.3429 | -0.7911 | -0.6838 | -0.7388 |
| Defecation | -0.8431 | -0.6937 | 0.1480 | -0.9607 | 1 | 0.5900 | 0.9297 | 0.8593 | 0.8967 |
| MDA | -0.9316 | -0.9908 | 0.8858 | -0.3429 | 0.5900 | 1 | 0.8458 | 0.9199 | 0.8863 |
| DK | -0.9818 | -0.9101 | 0.5017 | -0.7911 | 0.9297 | 0.8458 | 1 | 0.9872 | 0.9966 |
| KD | -0.9995 | -0.9644 | 0.6329 | -0.6838 | 0.8593 | 0.9199 | 0.9872 | 1 | 0.9969 |
| SHO | -0.9940 | -0.9408 | 0.5703 | -0.7388 | 0.8967 | 0.8863 | 0.9966 | 0.9969 | 1 |

VA – Vertical Activity; HA – Horizontal Activity; Groom. – Grooming

Horizontal activity is positively correlated with urination, but the correlation is weak and negatively correlated with grooming (high correlation) and defecation (medium correlation). Grooming indices are very weakly correlated with urination and defecation indices. And defecation and urination are highly negatively correlated with each other.

All indices of the concentration of peroxidation products in the brain homogenate of growing male rats, in all experimental groups have a high positive correlation with each other (Table 2).

Pearson's correlation between behavioural patterns and the level of lipid peroxidation products showed that vertical and horizontal activities have a high negative correlation with all lipid peroxidation products. Grooming has positive weak correlation with lipid peroxidation products except malonic aldehyde. The correlation between them is high. And on the contrary, indicators of defecation acts have high positive correlation with indicators of lipid peroxidation products, except for MDA. The correlation between them is weak. Acts of urination with lipid peroxidation products have a medium negative correlation, except for MDA. The correlation between them is very weak (Table 2).

Discussion

Diets that contain saturated lipids and sugar may contain high levels of trans fats, which can lead to the development of deviant behavior (Mohseni et al., 2021).

This is associated with the occurrence of the following sequence of events: lipid accumulation leads to an increase in the number of macrophages and local inflammation develops. Local inflammation

leads to the production of pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha and interleukin-6). The accumulation of macrophages and local inflammation creates conditions for the development of systemic inflammation (Cho et al., 2015; Adenan et al., 2020; Birkeland et al., 2023).

Also, hyperglycemia and hyperlipidemia increase the production of reactive oxygen species (ROS) (McMurray et al., 2016). High concentration of reactive oxygen species leads to the development or enhancement of oxidative stress, which can damage the basic components of any cell (proteins, lipids, carbohydrates and DNA) (Checa and Aran, 2020). The brain has a high metabolic rate and lower antioxidant levels, and therefore the brain is more vulnerable to oxidative stress (Sies and Jones, 2020). Therefore, oxidative stress is considered as one of the major factors in the development of brain-related diseases such as depression, Alzheimer's disease, schizophrenia, etc. (Bhatt et al., 2020; Rambaud et al., 2022).

Free-radical oxidation (FRO) of lipids and proteins is one of the processes regulating the metabolism of lipids, proteins and carbohydrates. Although in any cell of the organism there are substrates and initiators of triggering the process of FRO (polyene lipids and reactive oxygen species), in norm the content of FRO products in the organism is low. This is explained by the existence of a constantly functioning complex of mechanisms of the antioxidant defence system (ADS) (Esterbauer et al., 1990). Free radical oxidation is a number of processes occurring in several stages. In the process of free radical oxidation, a number of primary (ketodienes and conjugated dienes), intermediate (malonic dialdehyde) and final (Schiff bases) molecular products are formed, which



participate in the structural modification of biological membranes. Excess of free-radical oxidation products in the body leads to disruption of oxidative phosphorylation and microsomal oxidation in the cell (Kaya et al., 2007).

Ketodienes and conjugated dienes are primary products of free-radical oxidation of lipids, unstable, rapidly decompose to malonic dialdehyde. An increase in the content of ketodienes and conjugated dienes is an indicator of the intensity of free-radical oxidation processes (Corongiu and Banni, 1994).

Malonic dialdehyde is a secondary product of free-radical oxidation of lipids. Malonic dialdehyde has a damaging effect of the structural and functional state of biomembranes, which contributes to an increase in their permeability to calcium ions with the realisation of its damaging effect (Janero, 1990).

Schiff bases (the end product of free-radical oxidation of lipids and proteins) are formed by the interaction of malonic dialdehyde with proteins (Matela, 2020).

Our results have shown that in growing male rats on a diet high in fat and sucrose, the level of all indicators of lipid peroxidation increased significantly within 30 days. Particularly noteworthy is the sharp increase in Schiff bases, showing that the process acquires a chronic character already 30 days after the introduction of lipids and carbohydrates into the diet.

Antioxidants remove overproduced ROSs, inhibit the formation of ROSs, reducing the oxidation of cellular molecules, and alleviate oxidative stress (Mondin et al., 2016). Species of the genus *Stachys* have been studied as a good source of antioxidants, containing high amounts of flavonoids and polyphenols.

Thus, a study by Zarezade et al. (2021) found that the alcoholic extract of *Stachys pilifera* Benth had a strong anti-inflammatory effect, which may be related to the inhibition of lipid peroxidation (Zarezade et al., 2021).

In a study by Mansourian et al. (2019) proved that the aqueous-alcoholic extract of *Stachys pilifera* has hepatoprotective and antioxidant activity. This aqueous-alcoholic extract is able to reduce oxidative stress by inhibiting protein oxidation as well as increasing Glutathione peroxidase enzyme activity (Mansourian et al., 2019).

Our results showed that the use of *Stachys sieboldii* root powder, for 30 days together with a high-fat and sucrose diet, significantly reduced the levels of all free-radical oxidation products.

This is supported by other studies. For example, a study by Lee et al. (2020) found that *Stachys sieboldii* Miq root powder exhibited anti-obesity and antidyslipidaemic effects in rats following a high-fat and sucrose diet (Lee et al., 2020).

A study on the properties of *Stachys sieboldii* extract, as well as ginkgo extract, showed that together, they can protect the brain from learning and memory impairments, in ischaemic damage, through an antioxidant mechanism (Harada et al., 2015).

Likewise, a study on the properties of *Stachys sieboldii* Miq. root powder when supplemented with Sunsik showed that this combination could reduce serum and liver lipid components and improve lipid metabolism in rats with hyperlipidaemia induced by a high-fat and sucrose diet (Kang et al., 2018).

Also, previous studies have shown that some species of the genus *Stachys* have anxiolytic properties. A clinical study by Jadidi et al. (2023) showed that in combination with other stress relievers, *Stachys lavandulifolia* tea can be used as an adjunctive therapy for occupational stress in emergency physicians (Jadidi et al., 2023).

A study by Modarresi et al. (2020) proved the anxiolytic and antidepressant effects of an aqueous extract of *Stachys lavandulifolia* Vahl. in mice. It was suggested that this effect is mediated through GABA (Gamma aminobutyric acid) (Modarresi et al., 2020).

In a study by Jahani et al. (2019), the antidepressant activity of *Stachys lavandulifolia* alcohol extract was proved based on the data obtained in forced swim test and open field test. The antidepressant activity was attributed to the high flavonoid content of *Stachys lavandulifolia* alcohol extract (Jahani et al., 2019).

Our findings in the open-field test showed that both vertical and horizontal activity were reduced in growing males on a high-fat and sucrose diet. At the same time, the number of acts of grooming and defecation increased. Increased emotional reactivity is thought to be indicated by the presence of an autonomic defecation response and low locomotor activity. Defecation response under open field conditions reflects emotional reactivity and locomotor responses reflect motor and exploratory activity (Samarghandian et al., 2017).

Thus, our data show that growing males on a diet high in fat and sucrose have a change in the functioning of the nervous system, as the animals are apathetic, their locomotor activity and exploratory behaviour are reduced, and the level of emotional reactivity increases. Supplementation with *Stachys*



reduces the negative effect of the diet on the animals' organism - they significantly increase their exploratory behaviour, increase locomotor activity and decrease emotional reactivity.

A high negative correlation was found between the indicators of motor behavioural reactions and the concentration of lipid peroxidation products in the brain homogenate of growing rats. A high positive correlation was found between the indicators of emotionality (defecation) and the concentration of lipid peroxidation products in the brain homogenate of growing rats (except for the indicators of malonic dialdehyde levels).

Conclusion

Our study showed that diets high in fat and sucrose have a negative effect on growing male rats. They develop emotional reactivity and the animals show depressive behaviour. Also, in this group, the concentration of all lipid peroxidation indices increases in the brain homogenate. Introduction of *Stachys sieboldii* root powder into a high-fat and sucrose diet improved both behavioural reaction indices - the animals became more mobile and more emotional, as well as decreased concentrations of lipid peroxidation products in brain homogenate. Our study adds to the evidence that *Stachys sieboldii* species of the genus *Stachys* have antidepressant properties.

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Contribution of Authors

Pozdnyakova Y: Conceived idea, developed research methodology, edited the manuscript draft and approved and submitted final draft

Solyanov D, Tatina Y & Britko V: Conducted experiments, collected, analyzed and interpreted data and contributed and manuscript write up

Omarbekova N & Korshukova M: Statistical analysis and data interpretation and contributed and manuscript write up

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