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Anti-Metabolic Syndrome Effect of Trans-Cinnamic Acid

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Abstract.
The metabolic syndrome, also known as syndrome X or insulin resistance syndrome, is a global human health issue. It is associated with visceral obesity, insulin resistance, high blood pressure, hypoglycemia, hypocholesterolemia, and cardiovascular diseases. This article describes the anti-metabolic syndrome effect of several biologically active mixes that consisted of rutin, quercetin, and trans-cinnamic acid.
The experimental mixes differed in composition and ratio, with trans-cinnamic acid being the most abundant component. Mix 1 included rutin, quercetin, and trans-cinnamic acid (1:1:2), Mix 2 consisted of rutin and trans-cinnamic acid (1:3), Mix 3 was a combination of rutin, quercetin, and trans-cinnamic acid (4:1:15), Mix 4 consisted of quercetin and trans-cinnamic acid (3:1). The effective dose was 100.0 mg/kg for all samples. The hypocholesterolemic activity was studied on 48 male black C57Bl/6 mice with hypercholesterolemia induced by lipoprotein lipase inhibitor Poloxamer 407 (400.0 mg/kg). The hypoglycemic activity was determined *in vivo* on 42 white Wistar rats. Each rat was administered with an individual concentration of the experimental mix (effective doses: 100.0 mg/kg for the mixes, 5.0 mg/kg for glibenclamide, and 2 000.0 mg/kg for glucose). Blood was sampled from the tip of the tail to record the input data on glucose and total cholesterol.
The study revealed a reliable decrease ($p < 0.01$) in the area under curve for glucose concentration and time (Mix 3), which indicated hypoglycemic potential. All groups demonstrated a certain decrease in glucose, but it was statistically significant only in the animals that received Mix 3. All the mixes exhibited a reliable hypocholesterolemic effect. The tests on triglycerides and low-density lipoproteins revealed no statistically significant differences between the experimental groups. However, those treated with Mixes 2 and 3 demonstrated a trend towards lower triglycerides, and those that received Mixes 1 and 4 had a lower level of low-density lipoproteins.
Mixes 2 (rutin + trans-cinnamic acid, 1:3), 3 (rutin + quercetin + trans-cinnamic acid, 4:1:15), and 4 (quercetin + trans-cinnamic acid, 3:1) proved to be suitable for anti-metabolic syndrome bioactive additives.

Keywords. Trans-cinnamic acid, quercetin, rutin, metabolic syndrome, prevention, *in vivo*, mice

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Потенциал транс-коричной кислоты для профилактики метаболического синдрома

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Аннотация.
Метаболический синдром, также известный как синдром X или синдром резистентности к инсулину, является глобальной проблемой человечества, характеризующейся висцеральным ожирением, резистентностью к инсулину, высоким кровяным давлением, гипогликемией, гипохолестеринемией и сердечно-сосудистыми заболеваниями. Цель данного исследования – изучить потенциал профилактики метаболического синдрома с помощью смесей биологически активных веществ, состоящих из рутина (RUT), кверцетина (KVC) и транс-коричной кислоты (TKR-k), в составе которых преобладает TKR-k.
Объектами исследований послужили смеси биологически активных веществ, включающие транс-коричную кислоту, рутин и кверцетин в различных соотношениях: смесь № 1 – RUT:KVC:TKR-k в соотношении 1:1:2, смесь № 2 – RUT:TKR-k в соотношении 1:3, смесь № 3 – RUT:KVC:TKR-k в соотношении 4:1:15, смесь № 4 – KVC:TKR-k в соотношении 3:1.
Определение гипохолестеринемической активности исследуемых растворов биологически активных веществ в эффективной дозе 100,0 мг/кг осуществлялось на модельных объектах (самцах черных мышей), у которых моделировали гиперхолестеринемию введением ингибитора липопротеинлипазы – полоксамера P407 в эффективной дозе 400,0 мг/кг.
Определение гипогликемической активности проводилось *in vivo*. За час до запланированного времени введения смесей модельным объектам проводилась предварительная подготовка, в процессе которой каждую крысу взвешивали и подбирали индивидуально необходимую концентрацию смесей в эффективной дозе 100,0 мг/кг, глибенкламида – 5,0 мг/кг и глюкозы – 2000,0 мг/кг. Затем производили забор крови из кончика хвоста для протоколирования входных данных об уровне глюкозы и общего холестерина.
По результатам исследования выявлено достоверное снижение площади под кривой «концентрация глюкозы – время» (смесь № 3) ($p < 0,01$), что свидетельствует о гипогликемической активности. Зафиксирована тенденция снижения глюкозы во всех экспериментальных группах, однако достоверно уровень глюкозы в данной модели был снижен только в смеси № 3. Установлено, что смеси № 1–4 обладают выраженным достоверным гипохолестеринемическим эффектом. При определении уровня триглицеридов и липопротеинов низкой плотности статистически значимых различий между группами обнаружено не было, однако наблюдались тренды снижения триглицеридов в смесях № 2 и 3 и липопротеинов низкой плотности (смеси № 1 и 4).
Полученные результаты свидетельствуют о том, что целесообразнее использовать смеси № 2–4 как источники для создания биологически активных добавок, направленных на профилактику метаболического синдрома.

Ключевые слова. Транс-коричная кислота, кверцетин, рутин, метаболический синдром, профилактика, *in vivo*, мыши

Финансирование. Работа выполнена в рамках государственного задания по теме «Разработка биологически активных добавок, состоящих из метаболитов растительных объектов *in vitro*, для защиты населения от преждевременного старения» (проект FZSR-2024-0008) с использованием оборудования ЦКП «Инструментальные методы анализа в области прикладной биотехнологии» на базе КемГУ.

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Introduction

Premature aging is a global issue that modern healthcare seeks to prevent. All over the world, people strive to stay active and improve their life quality [1]. According to the Russian Monitoring of Economy and Health, consumers give priority to health and longevity. Yet, a serious health deterioration (20.2%) is registered in men aged 60–69 y.o. It reaches 35.6% in men aged 70–79 and 50.9% in 80-pusers. In women of the same age groups, the health deterioration data are 23.3, 39.4, and 60.7%, respectively [2]. As a result, the demand for dietary supplements is growing with each year [1].

The hallmarks of premature aging include early age-associated changes in appearance, memory impairment, fatigue and exhaustion, emotional instability, poor physical performance, and atherosclerosis [3].

Premature aging is strongly associated with the metabolic syndrome, which affects 25% global population and tends to increase with age [4].

The metabolic syndrome is a complex cluster of correlating metabolic disorders that increases the risk of cardiovas-cular diseases, type 2 diabetes, hypertension, and obesity [5]. The syndrome was first reported in the early 1920s but it was as late as in the 1980s that Gerald Reaven introduced the term Syndrome X to identify a group of risk factors that combined insulin resistance, impaired glucose tolerance, high triglycerides, low high-density lipoproteins, obesity, and arterial hypertension. A 2015 survey on obesity revealed that the number of obese people had doubled in 73 countries since 1980, resulting in the global epidemic of the metabolic syndrome [6]. Non-alcoholic fatty liver disease is another result of excessive fat and fructose in human diet.

The metabolic syndrome is mostly a human condition. The disruption of energy processing homeostasis has affected the vast majority of global population, and the numbers keep growing. Although the metabolic syndrome is no infectious disease, it represents a real epidemic threat to human health. Despite the diversity of pathological components and the damage depth, it affects predictable social strata, and its stages, i.e., onset, duration, complications, premature aging, and death, are quite well established [7].

The metabolic syndrome can be triggered at quite an early age by unfavorable living conditions, e.g., imbalanced diet, exposure to chemicals and some pharmaceutical products, etc. However, early preventive interventions may minimize the risks [8]. For instance, a balanced diet compensates for extreme conditions, providing macro- and micronutrients and bioactive compounds. Proper dieting is the most important factor: it reduces the risks of hypertension, hypoglycemia, hypocholesterolemia, disturbance in lipid metabolism, obesity, and inflammation, i.e., conditions that increase the risk of atherosclerosis, cardiovascular diseases, type 2 diabetes, etc. [4, 9]. A balanced diet requires functional foods that contain biologically active substances of plant origin. A systematic use of dietary supplements regulates metabolic reactions and restores the organism at various levels [10, 11].

Quercetin is a bioactive plant substance present in many fruits, vegetables, and green tea. It possesses antioxidant and anti-inflammatory properties. Quercetin reduces the risk of cardiovascular disease by improving the vascular system, as well as reducing inflammation and oxidative stress [5, 6].

Rutin, also known as vitamin P, is a bioflavonoid present in citrus fruits, vegetables, berries, green tea, and buckwheat. Its antioxidant properties make it possible to use it as a geroprotector, i.e., an anti-aging agent. Rutin reduces inflammation, which is known to trigger many age-related diseases. Its anti-inflammatory effect promoted good health. In addition, rutin strengthens capillaries and blood vessels, improving their elasticity and blood circulation [5].

Trans-cinnamic acid is a natural organic compound that participates in the synthesis of various chemical and pharmaceutical preparations, e.g., dietary supplements. Its range of biological activity includes antioxidant, antibacterial, and other properties. In [12], we reported the biopotential of trans-cinnamic acid isolated from the extract of Baikal skullcap (*Scutellaria baicalensis*). It was tested for hypoglycemic, hypocholesterolemic, and hepatotoxic activities *in vitro* and proved safe for human consumption. Trans-cinnamic acid demonstrated no cytotoxicity and could be recommended as an effective component for new dietary additives.

In this research, we focused on the synergistic effect of rutin, quercetin, and trans-cinnamic acid as a potential mix to be used in dietary additives aimed at preventing the metabolic syndrome.

The research objective was to study the hypoglycemic and hypocholesterolemic potential *in vivo* of biologically active mixes with trans-cinnamic acid as the main component.

Study objects and methods

The research involved mixes of biologically active trans-cinnamic acid, rutin, and quercetin.

The administration dose in the *in vivo* experiments was 100 mg/kg. The compositions were as follows:

Mix 1: rutin + quercetin + trans-cinnamic acid (1:1:2);

Mix 2: rutin + trans-cinnamic acid (1:3);

Mix 3: rutin + quercetin + trans-cinnamic acid (4:1:15);

Mix 4: quercetin + trans-cinnamic acid (3:1).

Mixes 1 and 3 contained more trans-cinnamic acid since our previous study had confirmed its biopotential [12]. Mixes 2 and 4 made it possible to evaluate the anti-metabolic syndrome potential of either rutin or quercetin with trans-cinnamic acid [12].

The hypoglycemic experiment involved 42 healthy white male Wistar rats (250 ± 15 g). The hypocholesterolemic test featured 48 healthy black male C57Bl/6 mice

(25 ± 10 g). Unlike females, males have no estrous cycle that affects susceptibility to etiological factors. These model objects were found most suitable for provoking the necessary health conditions.

The animals were kept at the vivarium complex of the Sechenov University, Institute of Regenerative Medicine The conditions were in line with State Standards GOST R 53434-2009 (December 02, 2009): Good Laboratory Practice; GOST 33216-2014 (July 01, 2016): Care and Use of Laboratory Rodents and Rabbits; GOST 33215-2014 (July 01, 2016): Care and Use of Laboratory Animals: Premises and Procedures; European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS N 123).

Hypoglycemic activity test. After a veterinary examination, the rats were quarantined for two weeks with access to food and water *ad libitum*. They were kept at 12:12 LD in isolated ventilated cages (VENT-BIO-2M, AWTech, Russia).

All the rats were deprived of food for 12 h before the administration, except for Group 1 animals (intact), which were not subjected to any medical intervention.

An hour before the administration, each rat was weighed to calculate the individual concentration of the experimental biologically active substances, sugar-reducing agent Glibenclamide (OZON Farmatsevtika, Russia, 5 mg/kg effective dose), and glucose (OAO Pharmstandard Leksredstva, Russia, 2 000 mg/kg effective dose). Each sample of individually measured substances was dissolved in 1 mL purified water [13, 14].

In line with SOP-IRM-LRV-110-01, each rat was given a serial number and labeled with carbol fuchsin (Fucorcin). Tail-tip blood samples revealed the input data on glucose and total cholesterol (SOP-IRM-LRV-112-01). To minimize pain and distress, the rats were sedated with sevoflurane by inhalation until depression of consciousness.

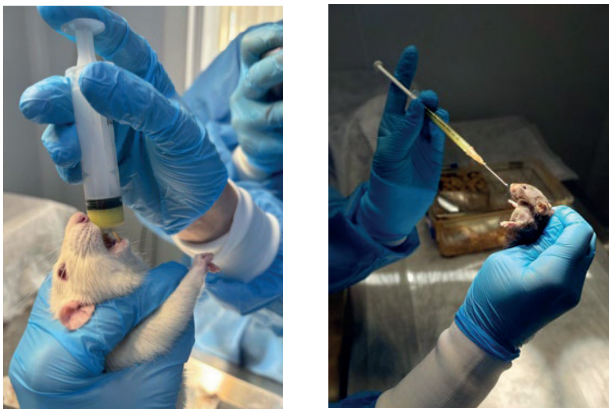
At the scheduled time points, Groups 2–6 were administered with the test substances via a gastric tube (Fig. 1a) in the numerical order as in Table 1. One hour later, the rats received an aqueous glucose solution via the same method. After that, glucose and total cholesterol were measured 30 min, 1, 1.5, 2, 4, and 6 h after the administration.

Glucose measurements involved a glucometer and Accu-Chek Performa test strips (Roche, Germany); the total cholesterol measurements involved an analyzer and EasyTouch test strips (Bioptik Technology, China). Microsamples of 50 μL were collected from the subcutaneous vein [15] for further validation using a ChemWell 2910 Biochemical and Enzyme Immunoassay Analyzer (Awareness Technology, USA). Plasma was obtained from whole blood by centrifugation at 2 000 g for 10 min and studied using a ChemWell 2910 Biochemical Analyzer with Glucose DDS and Cholesterol DDS reagents (DIAKON DS JSC, Russia).

In the end, the rats were euthanized with carbon dioxide in the EUTHANIZER Laboratory Animal Euthanasia Unit (AWTech, Russia) in line with SOP-IRM-LRV-109-01.

Hypocholesterolemic activity test. The black male C57Bl/6 mice underwent a veterinary examination and were quarantined for two weeks with access to food and water *ad libitum*. They were kept at 12:12 LD in isolated ventilated cages (VENT-BIO-2M, AWTech, Russia).

On experiment day 1, all the mice were assigned with a serial number (Table 2) and labeled with carbol fuchsin (Fucorcin) in line with SOP-IRM-LRV-110-01. After that, Groups 2–6 were weighed to calculate the individual concentration of the mixes (100 mg/kg effective dose) and lipoprotein lipase inhibitor Poloxamer 407 (400 mg/kg effective dose) (Fig. 1b). Each individual sample was dissolved in 1 mL purified water; Poloxamer 407 was dissolved in 1 mL physiological solution.



a b

Figure 1. Oral administration of test mixes via a gastric tube to Wistar rats (a) and C57Bl/6 mice (b)

Рисунок 1. Пероральное введение испытуемых смесей через зонд крысам линии Wistar (a); мышам линии C57Bl/6 (b)

Table 1. Hypoglycemic activity test on rats

Таблица 1. Дизайн исследования гипогликемической активности на крысах

Group №	Animals per group	Substance, dose
1	6	–
2	6	Purified water
3	6	Mix 1 (rutin + quercetin + trans-cinnamic acid, 1:1:2), 100 mg/kg
4	6	Mix 2 (rutin + trans-cinnamic acid, 1:3), 100 mg/kg
5	6	Mix 3 (rutin + quercetin + trans-cinnamic acid, 4:1:15), 100 mg/kg
6	6	Mix 4 (quercetin + trans-cinnamic acid, 3:1), 100 mg/kg
7	6	Glibenclamide, 5 mg/kg

Table 2. Hypocholesterolemic activity test on mice

Таблица 2. Дизайн исследования гипохолестеринемической активности испытуемых веществ

Group №	Animals per group	Substance, dose
1	8	–
2	8	Purified water
3	8	Mix 1 (rutin + quercetin + trans-cinnamic acid, 1:1:2), 100 mg/kg
4	8	Mix 2 (rutin + trans-cinnamic acid, 1:3), 100 mg/kg
5	8	Mix 3 (rutin + quercetin + trans-cinnamic acid, 4:1:15), 100 mg/kg
6	8	Mix 4 (quercetin + trans-cinnamic acid, 3:1), 100 mg/kg

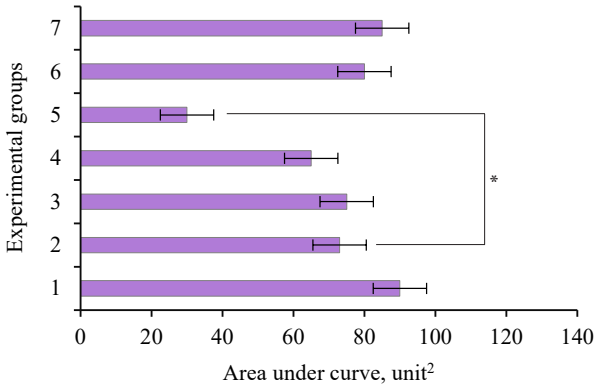


Figure 2. Area under curve for all groups of rats (* – $p < 0.01$)

Рисунок 2. График расчёта площади под кривой для всех групп крыс (* – при $p < 0,01$)

To simulate hypercholesterolemia, Groups 2–6 were injected intraperitoneally with 0.2 mL Poloxamer 407 solution (400 mg/kg effective) three times a week (Monday, Wednesday, Friday) for two weeks [16]. Group 1 consisted of intact animals that received no experimental treatment.

After the last administration, the mice were anesthetized with 12 mg/kg ZOLETIL 100 (VIRBAC, France) and 1 mg/kg Xyla (Interchemie, Netherlands) to take blood samples from the carotid artery into sterile test tubes. The glucose measurements were performed *in situ* using a glucometer and Accu-Chek Performa test strips (Roche, Germany). Plasma was obtained by centrifuging the samples at 2 000 g for 10 min and studied using a ChemWell 2910 Biochemical and Enzyme Immunoassay Analyzer (Awareness Technology, USA) with reagents for triglycerides, low-density lipoproteins, and cholesterol (AO DIAKON DS, Russia).

Statistical analysis. The data obtained underwent a statistical analysis using GraphPad Prism 8 (GraphPad Software, USA). The hypoglycemic and hypocholesterolemic effects were calculated with Area Under Curve. Significant differences between the test groups and the control were identified using the One-way ANOVA test followed by Dunnett's *post hoc* test ($p < 0.05$).

Results and discussion

The hypoglycemic test on rats followed the above-mentioned scheme. A single glucose loading revealed a reliable decrease in the area under the glucose concentration – time curve (Fig. 2) in Group 5 (Mix 3) ($p < 0.01$). This result indicated hypoglycemic activity.

Groups 6 (Mix 4) and 7 (gibenclamide) demonstrated a paradoxical increase in glucose levels, which could not be explained in this study and requires further research. The other groups showed no significant differences from the control.

Figure 3 illustrates individual glucose concentration – time graphs for each group.

Figure 3a shows the physiological fluctuations in glucose in the intact group. The average glucose level was 14 mmol/L at the beginning of the day, reached 24 mmol/L by the middle of the day, and gradually went down, with a minor physiological peak of 16 mmol/L in the evening.

Figure 3b shows the physiological change in glucose in the control group after the glucose loading preceded by fasting. The initial average glucose level was 9.5 mmol/L. After that, it rose to 20 mmol/L 30 min after the glucose loading and decreased steadily through all time points.

The experimental groups demonstrated a similar pattern, but the glucose increase after the glucose load was much lower.

Figure 3c illustrates the glucose fluctuations after the rats in Group 3 received Mix 1 and a glucose load preceded by fasting: the initial average glucose level of 8.2 mmol/L rose to 14.8 mmol/L after 30 min and reached 16.25 mmol/L after 1.5 h. There, it fluctuated until the time point of 2 h and then dropped down to 6.3 mmol/L in the evening.

Figure 3d shows the glucose measurements in Group 4 (Mix 2 + glucose load after fasting). The initial average glucose level was 7 mmol/L. After 30 min, it reached 14.5 mmol/L and fluctuate in this range up to the time point of 1.5 h only to drop down to 7 mmol/L in the evening.

Figure 3e demonstrates the glucose fluctuations in Group 5 (Mix 3 + glycose load after fasting). This group was different in that the glucose level remained practically the same (6–8 mmol/L) through all time points.

Figure 3f shows the average glucose level in Group 6 (Mix 4 + glucose load after fasting). The initial average glucose level was 8 mmol/L; it rose to 15 mmol/L 30 min after the glucose load, after which it decreased steadily through all time points.

Figure 3g shows glucose fluctuations in Group 7 after the rats were administered with glibenclamide and

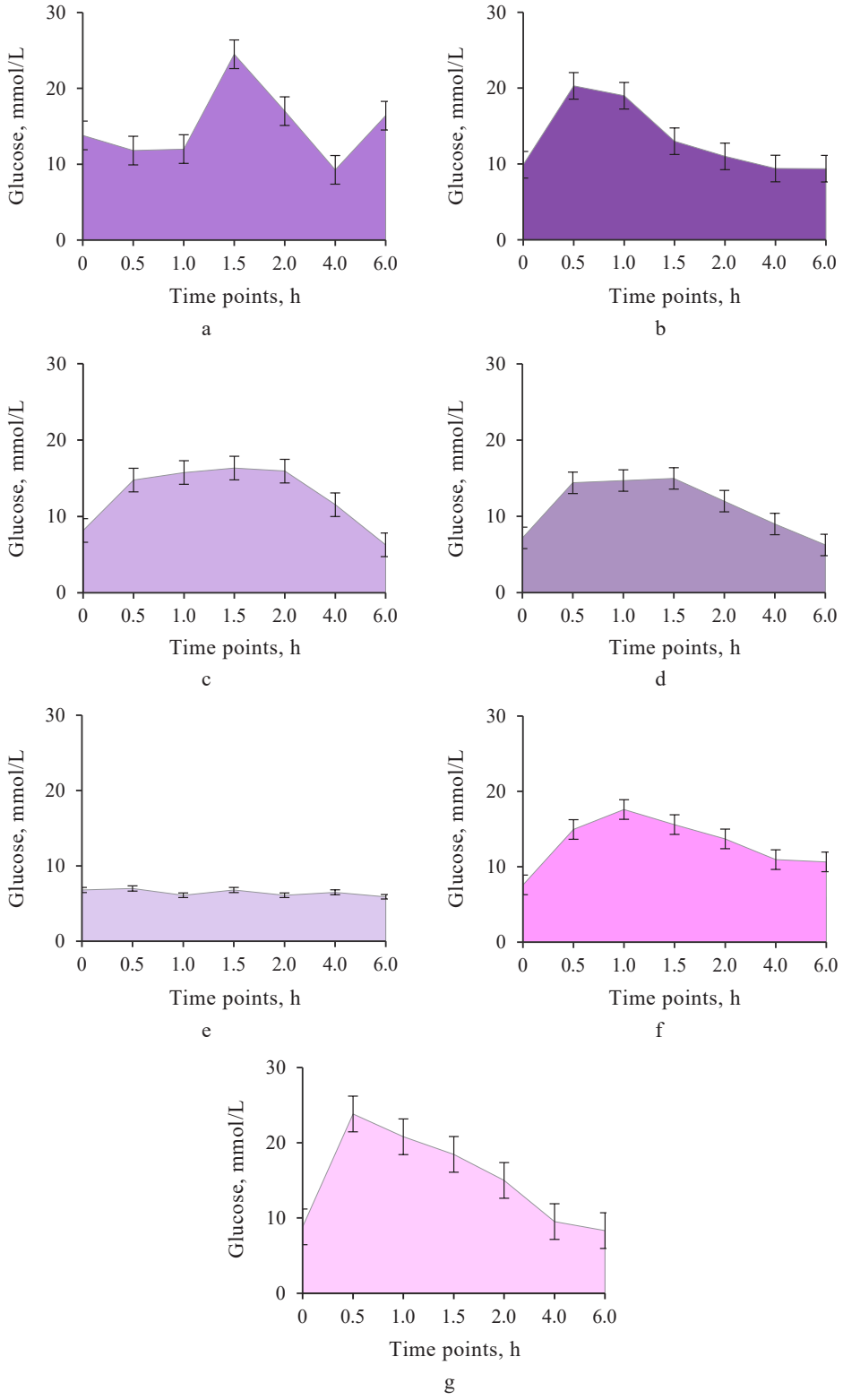


Figure 3. Glucose concentration – time curve for each group of rats: a – Group 1 (intact); b – Group 2 (purified water); c – Group 3, Mix 1 (rutin + quercetin + trans-cinnamic acid, 1:1:2); d – Group 4, Mix 2 (rutin + trans-cinnamic acid, 1:3); e – Group 5, Mix 3 (rutin + quercetin + trans-cinnamic acid, 4:1:15); f – Group 6, Mix 4 (quercetin + trans-cinnamic acid, 3:1); g – Group 7 (glibenclamide)

Рисунок 3. Графики «Концентрация глюкозы – время» для всех исследуемых групп: a – группа № 1 (без введения веществ); b – группа № 2 (вода очищенная); c – группа № 3, смесь № 1 (рутин + кверцетин + транс-коричная кислота, 1:1:2); d – группа № 4, смесь № 2 (рутин + транс-коричная кислота, 1:3), e – группа № 5, смесь № 3 (рутин + кверцетин + транс-коричная кислота, 4:1:15); f – группа № 6, смесь № 4 (кверцетин + транс-коричная кислота, 3:1); g – группа № 7 (глибенкламид)

received a glucose load preceded by fasting. The initial average glucose level was 9 mmol/L; it reached 24 mmol/L 30 min after the glucose load and then decreased steadily through all time points.

The glucose test (Fig. 2) proved that Mix 4 was capable of reducing the glucose levels.

Figure 4 illustrates the total cholesterol level – time correlation for each group.

Figure 4a shows the physiological fluctuations in total cholesterol in the intact group. The initial average cholesterol of 4.0 mmol/L dropped down to 3.4 mmol/L after 30 min. At the time point of 1.5 h, it rose to 4.5 mmol/L; by the evening, it went down to 3.5 mmol/L.

The control group revealed a physiological change in total cholesterol after the glucose load preceded by fasting (Fig. 4b). The initial average cholesterol was 5.8 mmol/L, but 1 h after the glucose load, it dropped to 3.2 mmol/L. Then, it started rising and reached 3.8 mmol/L by the time point of 4 h. After that, it demonstrated a stable decrease and dropped down to 3.0 mmol/L at the time point of 6 h.

Figure 4c shows the total cholesterol in Group 3, where the mice received Mix 1 and a glycose load preceded by fasting. The fluctuations detected stayed within the limits of single values. The average total cholesterol was 5.6 mmol/L during the day and 5.46 mmol/L in the evening.

Figure 4d demonstrates the average total cholesterol in Group 4 (Mix 2 + glycose load after fasting). The fluctuations remained within the limits of single values. The average total cholesterol was 5.2 mmol/L early in the day and dropped down to 4.4 mmol/L in the evening.

Figure 4e illustrates the total cholesterol in Group 5 (Mix 3 + glycose load after fasting). The fluctuations stayed within the limits of single values. The average total cholesterol was 4.8 mmol/L early in the day and increased steadily to 4.9 mmol/L.

Figure 4f demonstrates the fluctuations in total cholesterol in Group 6 (Mix 4 + glucose load after fasting). The initial average total cholesterol was 5.4 mmol/L; 30 min after the load, it peaked at 5.8 mmol/L. Two hours after the glucose load, it decreased slightly to 5.3 mmol/L and then increased steadily to reach 5.7 mmol/L by the evening.

Figure 4g describes the total cholesterol in the mice from Group 7 that received glibenclamide after the glucose load preceded by fasting. Early in the day, it was 4.6 mmol/L, then peaked up to 5.2 mmol/L at the time point of 30 min. By the evening, it gradually decreased to 4.23 mmol/L.

In general, Figure 4 shows quite clearly that the mixes of biologically active substances had virtually no effect on total cholesterol.

Hypocholesterolemic activity of biologically active mixes. Cholesterol is a vital component that participates in digestion and other processes. It is mostly synthesized in the liver from fats, glucose, and amino acids, but some part enters the body with food. High cholesterol may lead to stroke or myocardial infarction, depending on the area affected [17]. High cholesterol is a major risk factor associated with low life expectancy. Therefore, medications aimed at curbing this component of the metabolic syndrome require serious research.

The analysis of the hypocholesterolemic activity of the biologically active mixes followed the protocol described above.

Table 3 shows the survival rate for each group of animals. All glucose measurements were performed from 9 a.m. to 9 p.m. If an animal died at night, rigor mortis made glucose measurements impossible. The mice were randomly selected to perform one-time spontaneous glucose measurements and test the hypothesis that the biologically active mixes could reduce the glucose level until lethargy, apathy, etc.

Figure 5 shows the average glucose level in the hypercholesterolemia model induced by Poloxamer 407 after the experimental mixes were administered at a concentration of 100.0 mg/kg. The glucose level (Fig. 5) increased in the control group relative to the intact group, which is consistent with other reports [18]. All the experimental groups had lower glucose than the control, which means that all the mixes had a potential hypoglycemic effect. However, the decrease was statistically significant ($p < 0.01$) only in Group 5, where the animals received Mix 3. The data obtained confirmed the results of the hypoglycemic test on rats.

Figure 6 illustrates the average total cholesterol across all groups of mice administered with experimental mixes at a concentration of 100.0 mg/kg. The total cholesterol test (Fig. 6) revealed that the mice developed hypercholesterolemia upon receiving 400 mg/kg Poloxamer 407. In the intact group, the cholesterol level was below the control values ($p < 0.05$). The low cholesterol in Groups 3 (Mix 1), 4 (Mix 2), 5 (Mix 3), and 6 (Mix 4) relative to the control (Group 2) indicated a pronounced hypocholesterolemic activity of the corresponding biologically active mixes. No significant differences were detected between the groups ($p = 0.0983$).

Figure 7 shows the average triglyceride values for all the groups of mice that received the experimental solutions at a concentration of 100.0 mg/kg.

The triglyceride test (Fig. 7) revealed no statistically significant differences between the groups after the treatment with poloxamer P407 and the experimental mixes. However, the control group demonstrated a trend to increase relative to the intact group whereas the experimental groups had lower total triglyceride, especially in Groups 4 (Mix 2) and 5 (Mix 3), which complemented the results described above.

Figure 8 shows the average low-density lipoprotein across the groups of mice that received the experimental substance mixes at a concentration of 100.0 mg/kg.

The low-density lipoprotein test in the hypercholesterolemia model (Fig. 8) revealed no statistically sig-

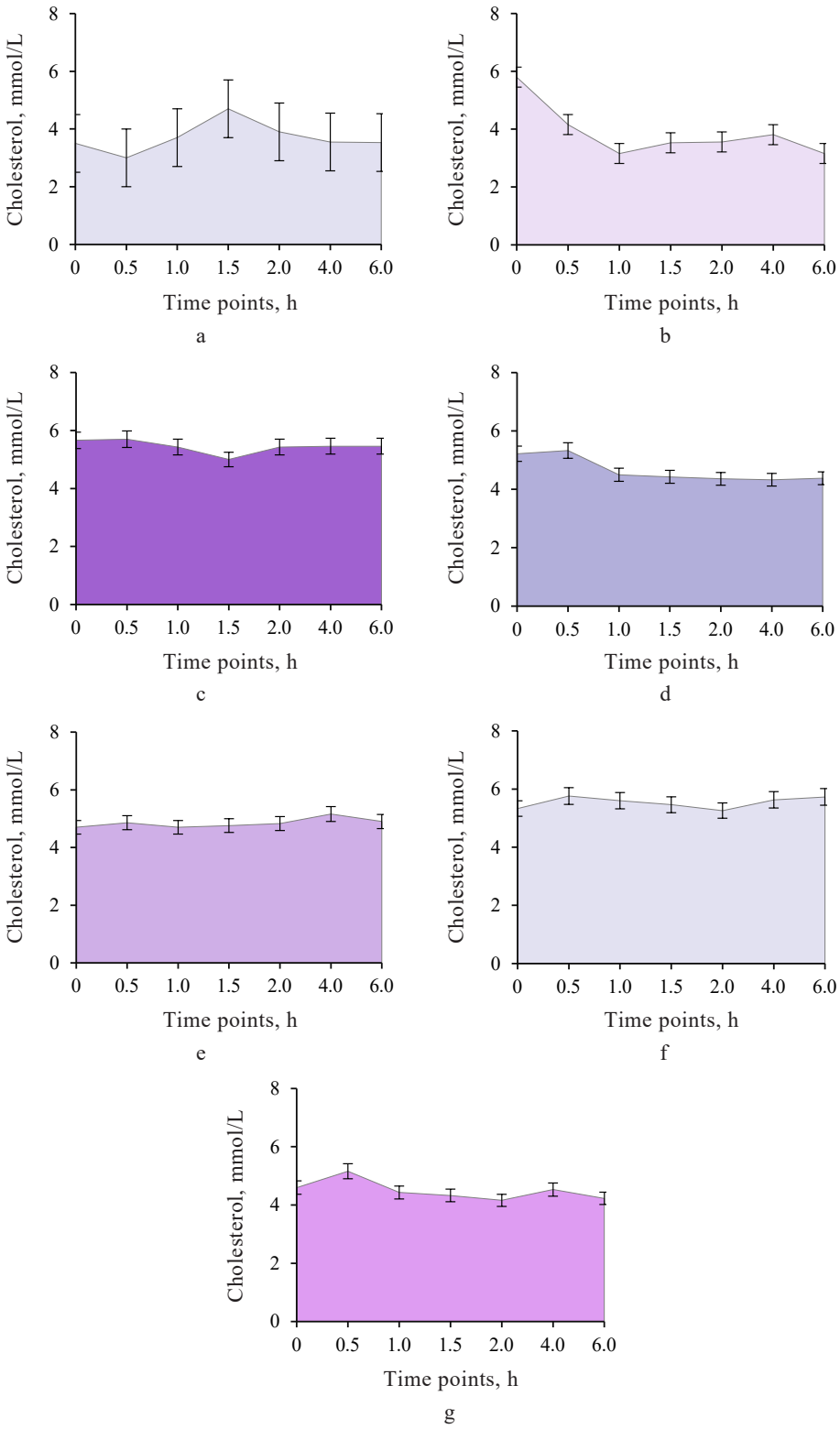


Figure 4. Cholesterol – time curve for each group of mice: a – Group 1 (intact); b – Group 2 (purified water); c – Group 3, Mix 1 (rutin + quercetin + trans-cinnamic acid, 1:1:2); d – Group 4, Mix 2 (rutin + trans-cinnamic acid, 1:3); e – Group 5, Mix 3 (rutin + quercetin + trans-cinnamic acid, 4:1:15); f – Group 6, Mix 4 (quercetin + trans-cinnamic acid, 3:1); g – Group 7 (glibenclamide)

Рисунок 4. Графики «Уровень холестерина – время»: a – группа № 1 (без введения веществ); b – группа № 2 (вода очищенная); c – группа № 3, смесь № 1 (рутин + кверцетин + транс-коричная кислота, 1:1:2); d – группа № 4, смесь № 2 (рутин + транс-коричная кислота, 1:3); e – группа № 5, смесь № 3 (рутин + кверцетин + транс-коричная кислота, 4:1:15); f – группа № 6, смесь № 4 (кверцетин + транс-коричная кислота, 3:1); g – группа № 7 (глибенкламид)

Table 3. Survival rate and causes of death in each group of mice (eight mice per group)

Таблица 3. Данные о выживаемости и причинах смерти в каждой из экспериментальных групп мышей (всего в группе восемь животных)

Group №	Survived	Died
1	8	–
2	8	–
3	5	1 (aspiration pneumonia); 2 (Day 11, Day 14, 2 h before exclusion; cause undetected; severe lethargy and apathy the day before)
4	4	4 (aspiration pneumonia)
5	7	1 (Day 7, at night)
6	8	–

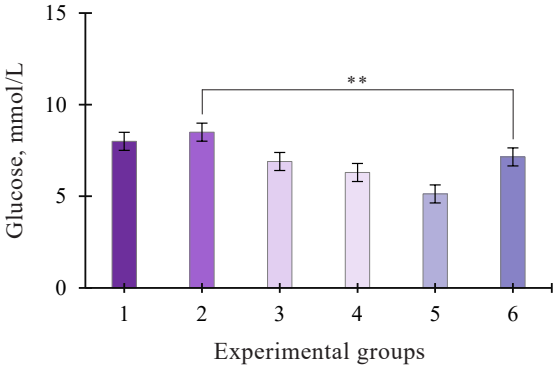


Figure 5. Glucose concentration across groups after the experiment (** – $p < 0.01$; mean values ± standard deviation)

Рисунок 5. График значений концентрации глюкозы во всех группах после окончания эксперимента (** – при $p < 0,01$; отражены средние значения ± стандартное отклонение)

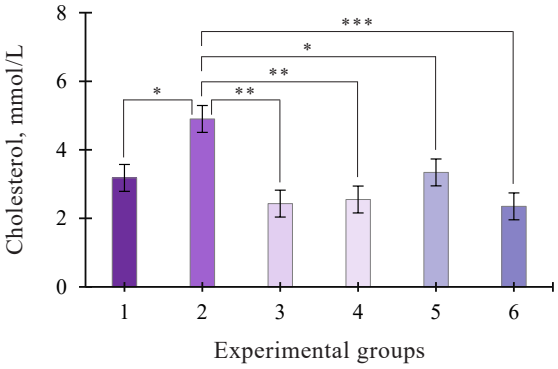


Figure 6. Total cholesterol across groups after the experiment (* – $p < 0.1$; ** – $p < 0.01$; *** – $p < 0.001$; mean values ± standard deviation)

Рисунок 6. График значений уровня общего холестерина во всех группах после окончания эксперимента (* – при $p < 0,1$; ** – при $p < 0,01$; *** – при $p < 0,001$; отражены средние значения ± стандартное отклонение)

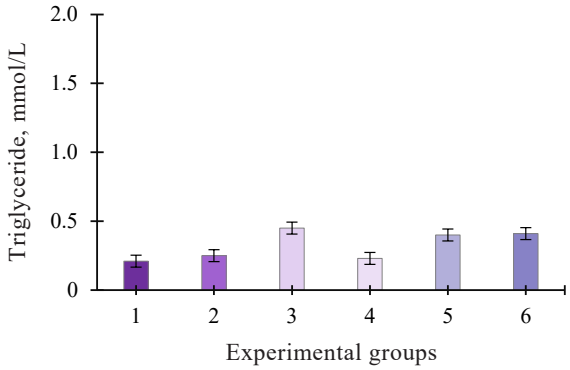


Figure 7. Triglyceride across groups after the experiment (mean values ± standard deviation)

Рисунок 7. График значений уровня ТГД во всех группах после окончания эксперимента (отражены средние значения ± стандартное отклонение)

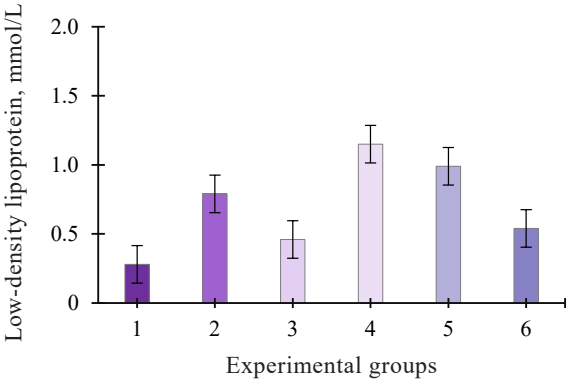


Figure 8. Low-density lipoprotein across groups after the experiment (mean values ± standard deviation)

Рисунок 8. График значений уровня ЛПНП во всех группах после окончания эксперимента (отражены средние значения ± стандартное отклонение)

nificant differences between the groups. However, the abovementioned trends persevered: the control group had a higher low-density lipoprotein level than the intact group. The results indicated the effect of Poloxamer 407 on the overall lipid profile. However, only Groups 4 (Mix 2) and 6 (Mix 4) demonstrated a trend towards a lower low-density lipoprotein level.

The *in vivo* study on rodents proved that Mix 3 was the most effective glucose reducer, regardless of the model object, for both hypoglycemia and hypocholesterolemia. The hypocholesterolemia model revealed no mix that could reduce all four indicators, i.e., glucose, total cholesterol, low-density lipoprotein, and triglycerides. Yet, Mix 3 proved able to reduce at least three indicators, i.e., glucose, total cholesterol, and triglycerides. Mix 1 reduced total cholesterol and low-density lipoprotein; Mixes 2 and 4 reduced total cholesterol and triglycerides.

Mix 1 (rutin + quercetin + trans-cinnamic acid, 1:1:2) could reduce cholesterol and low-density lipoprotein.

The hypocholesterolemic activity test took the lives of three animals: one died from aspiration pneumonia, the other two died from unspecified cause, with severe lethargy and apathy the day before death.

Mix 2 (rutin + trans-cinnamic acid, 1:3) reduced cholesterol and triglycerides, with four animals dead from aspiration pneumonia.

Mix 3 (rutin + quercetin + trans-cinnamic acid, 4:1:15) was able to reduce glucose, cholesterol, and triglycerides, with one animal dead (cause unidentified).

Mix 4 (quercetin + trans-cinnamic acid, 3:1) increased glucose levels while reducing cholesterol and low-density lipoprotein.

Based on the *in vivo* bioactivity, Mixes 1, 3, and 4 proved relevant for further component analysis to reveal the lethal dose and the causes of death.

We already reported the hypoglycemic and hypocholesterolemic activities of trans-cinnamic acid in [12, 19]. The study *in vivo* involved female Sprague Dawley rats (*Rattus* sp.), male Wistar rats (*Rattus* sp.), male mice (*Mus musculus*), and CD-1 female mice (*Mus musculus*). Trans-cinnamic acid exhibited a proinflammatory effect in acute inflammation induced by *λ*-carrageenan. It reduced the mass of granulation tissue by 20% in a statistically significant manner but did not affect the exudative reaction in proliferative inflammation caused by cotton swabs implanted under the skin. However, when administered into the stomach of rats with alloxan-induced diabetes at 50.0 and 100.0 mg/kg for 7 days, trans-cinnamic acid had no effect on body weight and demonstrated no hypocholesterolemic activity.

Other authors reported that that trans-cinnamic acid isolated from Baikal skullcap (*Scutellaria baicalensis*) facilitated the survival of nematodes (*Caenorhabditis elegans*) subjected to oxidative stress, which indicates its geroprotective potential [20, 21].

Another publication [22] mentions the mechanisms of action of trans-cinnamic acid against non-alcoholic fatty liver disease in rats *in vivo*. Forty-eight rats were split into two groups: one spent a week on a normal diet and the other spent a week on a diet high in fat and fructose. The latter were divided into control groups that received trans-cinnamic acid and pioglitazone. The rats on the fat-and-fructose diet gained more weight than the rats in the trans-cinnamic acid group. Their body weight went down with the trans-cinnamic acid treatment. The rats that received trans-cinnamic acid had lower platelet counts. The trans-cinnamic acid treatment reduced the body weight, fat mass, and lipids in obese rats, as well as liver markers and anti-inflammatory cytokinin TNF-*α*. Trans-cinnamic acid with its ability to reduce inflammation via TNF-*α* makes proved effective against non-alcoholic fatty liver disease. Another *in vivo* study [23] showed that receptors and gene expression could reduce both inflammation of adipose tissue and metabolic dysfunction.

Shah *et al.* [24] described a mix of nicotinamide and trans-cinnamic acid that stimulated the survival of pancreatic β-cells in combination with insulin secretion via the ERK1/2 signaling pathway in an animal apoptosis model. The Wistar rats received daily injections of nicotinamide and trans-cinnamic acid for three days, followed by streptozotocin. On Day 3, the scientists detected an acute effect on blood glucose and serum insulin, and as survival potential against streptozotocin-induced apoptosis, in all experimental groups.

Yazdi *et al*. [25] reported a decrease in DPP4 expression in both normal and high-glucose HepG2 cells treated with cinnamic acid. In hyperglycemic cells, the highest activity belonged to 50 mg/mL trans-cinnamic acid.

The neuroendocrine function of visceral fat triggers the development of insulin resistance, oxidative stress endothelial dysfunction, and vascular disorders as parts of the metabolic syndrome. In [26], described the effect of a phytoadaptogen complex on the endocrine and immune systems with a change in the content and synthesis of biologically active substances, i.e., hormones, cytokines, and neurotransmitters. In addition, the complex reduced the low-intensity chronic inflammation in the metabolic syndrome, thus correcting microcirculation disorders due to stable antioxidant, stress-limiting, and anti-inflammatory effects.

Patients with the metabolic syndrome may be genetically predisposed to obesity and exhibit proinflammatory responses. Adipose tissue secretes countless autoimmune components (adipokines) into the circulation, including tumor necrosis factor-*α* (TNF-*α*), leptin, adiponectin, resistin, monocyte chemoattractant protein-1, adipocyte-type fatty acid binding protein, etc. The metabolic syndrome is closely associated with oxidative stress and inflammation, with increased TNF-*α* expression in adipose tissue being associated with obesity-induced insulin resistance [27].

Conclusions

The hypoglycemic tests revealed a trend towards a decrease in glucose across all experimental groups, which indicates a potential hypoglycemic effect of all the biologically active mixes. However, the only statistically significant decrease was observed in the group treated with Mix 3, which consisted of rutin, quercetin, and trans-cinnamic acid (4:1:15). Mix 2 (rutin + transcinnamic acid, 1:3) and Mix 4 (quercetin + trans-cinnamic acid, 3:1) demonstrated a trend towards lower low-density lipoprotein.

Since trans-cinnamic acid had no hypocholesterolemic effect in previous studies, Mixes 2, 3, and 4 could be potential sources for new dietary supplements against the metabolic syndrome.

Contribution

D.Yu. Chekushkina was responsible for data curation, project administration, validation, visualization, original draft, and proofreading. I.S. Milentyeva developed the research concept, supervised the project,

and proofread the draft. A.M. Fedorova provided verification and editing. S.V. Kovalenko processed the data and wrote the original draft. O.G. Altshuler designed the methodology and proofread the manuscript. L.M. Aksenova provided verification and editing.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

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Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

References

1. Spivak IM, Slizhov PA, Pleskach NM, Nyrov VA, Panferov EV, *et al*. Geroprotectors against accelerated and natural aging. Health – The Base of Human Potential: Problems and Ways to Solve Them: Proceedings XIII Annual All–Russian Research and practical Conf. with Intern. Participation, 2018;13(1):133–143. (In Russ.)

2. Lemieux I, Després J-P. Metabolic Syndrome: Past, present and future. Nutrients. 2020;12(11):3501. https://doi.org/10.3390/nu12113501

3. Pristrom MS, Shtonda MV, Semenenkov II. A look at the problem of premature aging: Approaches to prevention. General Medicine: Journal of Scientific and Practical Therapy. 2021;(1):5–24. (In Russ.) https://elibrary.ru/MSIOAR

4. García-García FJ, Monistrol-Mula A, Cardellach F, Garrabou G. Nutrition, bioenergetics, and metabolic syndrome. Nutrients. 2020;12(9):2785. https://doi.org/10.3390/nu12092785

5. Vesnina AD, Milentyeva IS, Le VM, Fedorova AM, Altshuler OG, *et al*. Quercetin isolated from *Hedysarum neglectum* Ledeb. as a preventer of metabolic diseases. Foods and Raw Materials. 2025;13(1):192–201. https://doi.org/10.21603/2308-4057-2025-1-633

6. Vesnina A, Milentyeva I, Minina V, Kozlova O, Asyakina L. Evaluation of the *in vivo* anti-atherosclerotic activity of quercetin isolated from the hairy roots of *Hedysarum neglectum* Ledeb. Life. 2023;13(8):1706. https://doi.org/10.3390/life13081706

7. Kim J-E, Kim J-S, Jo M-J, Cho E, Ahn S-Y, *et al*. The Roles and associated mechanisms of adipokines in development of metabolic syndrome. Molecules. 2022;27(2):334. https://doi.org/10.3390/molecules27020334

8. Alemany M. The Metabolic syndrome, a human disease. International Journal of Molecular Sciences. 2024;25(4):2251. https://doi.org/10.3390/ijms25042251

9. Vesnina A, Prosekov A, Atuchin V, Minina V, Ponasenko A. Tackling atherosclerosis via selected nutrition. International Journal of Molecular Sciences. 2022;23(15):8233. https://doi.org/10.3390/ijms23158233

10. Hsu C-N, Hou C-Y, Hsu W-H, Tain Y-L. Early-life origins of metabolic syndrome: Mechanisms and preventive aspects. International Journal of Molecular Sciences. 2021;22(21):11872. https://doi.org/10.3390/ijms222111872

11. Aslanova MA, Vasilevskaya ER, Kotenkova EA, Chernukha IM, Fedulova LV, *et al*. Scientific approaches to the selection of biologically active substances of animal and plant origin for metabolic syndrome correction. Meat Industry. 2020;(12):18–21. (In Russ.) https://doi.org/10.37861/2618-8252-2020-11-18-21

12. Chekushkina DYu, Milentyeva IS, Le VM, Prosekov AYu, Proskuryakova LA. The biopotential of trans-cinnamic acid. Bulletin of the South Ural State University. Series: Food and Biotechnology. 2024;12(2):87–95. (In Russ.) https://doi.org/10.14529/food240210

13. Pharmacopoeia.rf. Art.1.2.3.0019.15: Spectrophotometry in sugar studies. Ministry of Health of the Russian Federation. Available from: https://pharmacopoeia.ru/ofs-1-2-3-0019-15-opredelenie-saharov-spektrofotometricheskim-metodom/

14. Mah PT, Laaksonen T, Rades T, Aaltonen J, Peltonen L, *et al*. Unravelling the relationship between degree of disorder and the dissolution behavior of milled glibenclamide. Molecular Pharmaceutics. 2014;11(1):234–242. https://doi.org/10.1021/mp4004145

15. Caron A, Lelong C, Bartels T, Dorchies O, Gury T, *et al*. Clinical and anatomic pathology effects of serial blood sampling in rat toxicology studies, using conventional or microsampling methods. Regulatory Toxicology and Pharmacology. 2015;72(3):429–439. https://doi.org/10.1016/j.yrtph.2015.05.022

16. Lee U, Kwon MH, Kang HE. Pharmacokinetic alterations in poloxamer 407-induced hyperlipidemic rats. Xenobiotica. 2019;49(5):611–625. https://doi.org/10.1080/00498254.2018.1466212

17. Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486–97. https://doi.org/10.1001/jama.285.19.2486

18. Johnston TP, Edwards G, Koulen P. Synergism of mechanisms underlying early-stage changes in retina function in male hyperglycemic *db/db* mice in the absence and presence of chemically-induced dyslipidemia. Scientific Reports. 2023; 13:17347. https://doi.org/10.1038/s41598-023-44446-3

19. Fedorova AM, Dyshlyuk LS, Milentyeva IS, Loseva AI, Neverova OA, *et al*. Geroprotective activity of trans-cinnamic acid isolated from the Baikal skullcap (*Scutellaria baicalensis*). Food Processing: Techniques and Technology. 2022; 52(3):582–591. (In Russ.) https://doi.org/10.21603/2074-9414-2022-3-2388

20. Faskhutdinova ER, Dmitrieva AI, Milentyeva IS. Effect of trans-cinnamic acid on oxidative stress resistance in *Caenorhabditis elegans*. Innovative development of agro-industrial, chemical, and forestry complexes and sustainable nature management. 2022. pp. 147–150. (In Russ.)

21. Milentyeva IS, Fedorova AM, Larichev TA, Altshuler OG. Biologically active compounds in *Scutellaria baicalensis* L. callus extract: Phytochemical analysis and isolation. Foods and Raw Materials. 2023;11(1):172–186. https://doi.org/10.21603/2308-4057-2023-1-564

22. Solanki N, Patel R. Unraveling the mechanisms of trans-cinnamic acid in ameliorating non-alcoholic fatty liver disease. American Journal of Translational Research. 2023;15(9):5747–5756.

23. Bishehsari F, Voigt RM, Keshavarzian A. Circadian rhythms and the gut microbiota: From the metabolic syndrome to cancer. Nature Reviews Endocrinology. 2020;16:731–739. https://doi.org/10.1038/s41574-020-00427-4

24. Shah SAR, Khan MI, Jawaid H, Qureshi U, Ul-Haq Z, *et al*. Nicotinamide-cinnamic acid cocktail exerts pancreatic β-cells survival coupled with insulin secretion through ERK1/2 signaling pathway in an animal model of apoptosis. DARU Journal of Pharmaceutical Sciences. 2021;29:483–492. https://doi.org/10.1007/s40199-021-00412-w

25. Yazdi M, Nafari A, Azadpour M, Alaee M, Hadipour Moradi F, *et al*. Protective effects of cinnamic acid against hyperglycemia induced oxidative stress and inflammation in HepG2 cells. Reports of Biochemistry and Molecular Biology. 2023;12(1):1–12. https://doi.org/10.52547/rbmb.12.1.1

26. Dzampaeva ZhV, Datieva FS, Takoeva EA, Nartikoeva MI. Correction of chronic systemic low-intensity inflammation in metabolic syndrome with complex phytoadaptogens. Journal of New Medical Technologies. 2022;(6):101–107. (In Russ.) https://doi.org/10.24412/2075-4094-2022-6-3-7

27. Alshehri FF. Understanding the prevalence, progression, and management of metabolic syndrome in Saudi Arabia. Saudi Medical Journal. 2023;44(10):973–986. https://doi.org/10.15537/smj.2023.44.10.20230450