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ASSESSMENT OF SAFETY AND TOLERABILITY OF HYBRID GEL ORAL ADMINISTRATION IN AN EXPERIMENT ON WISTAR RATS

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oleogel, hydrogel, hybrid gel, safety, tolerability, hematological parameters, biochemical parameters, linear Wistar rats

ABSTRACT

Due to the negative health effects such as cardiovascular diseases, diabetes and obesity, the consumers avoid foods high in saturated fatty acids. For this reason, one of the main goals of the food industry is to develop the substitutes for solid fats rich in unsaturated fatty acids. Recent studies have shown that oleogels can successfully replace saturated fats in various foods such as cakes, biscuits, meat products, chocolate and ice cream. We have developed a hybrid gel in which oleogel is made up from the composition of hydrogel and oleogel in a ratio of 5:95. The hydrogel is obtained from a 2 % solution of sodium alginate in combination with oleogel made from grape seed oil and beeswax in a concentration of 20 %. The safety assessment of the food hybrid gel was carried out on laboratory animals (linear Wistar rats). The animals were split into three groups to conduct the research. The rats of the first group got per oral injection with the gel being researched at a dose of 1 g of hybrid gel / kg of rat weight, the second group received three-fold increased dose — 3 g of hybrid gel / kg of rat weight for 30 days, the third control group was fed with a standard diet. Based on the results obtained, the safety and tolerability of oral administration of an edible hybrid gel based on oleogel made up from beeswax in Wistar rats was defined. The prospects for creating edible hybrid gels with oleogel from beeswax seem promising, as they solve current dietary and health issues while providing functional and sensory benefits in food formulations.

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ОЦЕНКА БЕЗОПАСНОСТИ И ПЕРЕНОСИМОСТИ ПЕРОРАЛЬНОГО ПРИМЕНЕНИЯ ГИБРИДНОГО ГЕЛЯ В ЭКСПЕРИМЕНТЕ НА КРЫСАХ ЛИНИИ WISTAR

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КЛЮЧЕВЫЕ СЛОВА:
олеогель, гидрогель, гибридный гель, безопасность, переносимость, гематологические показатели, биохимические показатели, крысы линии Wistar

АННОТАЦИЯ

Негативные последствия для здоровья, такие как сердечно-сосудистые заболевания, диабет и ожирение, в том числе связанные с чрезмерным потреблением продуктов с высоким содержанием насыщенных жиров, призывают пищевую промышленность разрабатывать новые технологии заменителей твердых жиров, богатых ненасыщенными жирными кислотами. Недавние исследования показали, что олеогели могут успешно заменять насыщенные жиры в различных пищевых продуктах, таких как торты, печенье, мясные изделия, шоколад и мороженое. Нами разработан способ производства гибридного геля из смеси гидрогеля и олеогеля в соотношении 5:95, соответственно. Гидрогель представляет собой 2 %-ый раствор альгината натрия. Олеогель был изготовлен из масла виноградных косточек и пчелиного воска в концентрации 20 %. Оценку безопасности пищевого гибридного геля осуществляли на лабораторных животных (крысы линии Wistar). Для проведения исследований было сформировано три группы животных. Крысам первой группы орально вводили исследуемый гель в дозе 1 г гибридного геля/кг массы крысы, второй в трехкратно увеличенной дозе — 3 г гибридного геля/кг массы крысы в течение 30 дней, третья группа контрольная — содержалась на стандартном рационе. На основании полученных результатов была установлена безопасность и переносимость перорального применения съедобного гибридного геля на основе олеогеля из пчелиного воска на крысах линии Wistar. Перспективы создания съедобных гибридных гелей с олеогелем из пчелиного воска представляются многообещающими, поскольку они решают текущие диетические проблемы и проблемы со здоровьем, обеспечивая при этом функциональные и сенсорные преимущества в рецептурах пищевых продуктов.

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1. Introduction

Restrictions imposed at the legislative level on application of trans and saturated fats [1,2], the growing concern of the scientific community and the food consumers about the negative impact of such types of fats on their health [3,4] urge the food industry to develop new technologies that contribute to the reformulation and modification of fat-containing food products composition. A wide range of well-studied fatty acids ensures that fat-based structuring is fairly versatile. Careful selection of the triglyceride composition in combination with proper processing will make it possible to create fat-containing food systems with a various range of hardness and melting points [5]. The production of food oleogels (oleogelation) is currently one of the safest technological methods to reduce the content or completely replace saturated and trans fats in food products [6,7]. In addition, the regulation of properties by adjusting the composition and combinations of gel systems by various combinations of hydrogels and oleogels leads to the formation of so-called hybrid gels (or bigels) with both hydrophilic and lipophilic properties [8,9].

Edible oleogels and hybrid gels are developed in many methods and are used in the food industry in the production of various food products: spreads [10–13], pastry [14,15] and confectionery products [16,17], cheese [18,19] and meat products [20–24]. As a result of the research, the developers have obtained acceptable reformulated food products with similar technological and rheological properties as the traditional food products or even food products with improved technical functionality. By their nature, oleogels are a variety of soft materials, that range from viscoelastic liquid to solid matter, consisting of an organic liquid (vegetable oil) and a gelling component, an organogelator capable of producing the formation of an ordered supramolecular structure and structuring of vegetable oil [25]. In most cases, low molecular weight amphiphilic molecules are responsible for gelation [26], which provide thermoregulative properties to the gel [27,28]. However, some biomacromolecules attract special attention, such as natural waxes, gums and biopolymers with gelling properties in oily media, [29,30].

Currently, recommendations for an optimal fatty acid intake profile often focus on limiting saturated fat intake to less than 10 % of the caloric content, thus maintaining trans fatty acid intake at the lowest possible level, and replacing saturated fats with the foods rich in mono- and polyunsaturated fatty acids [1]. However, this is a definite challenge for the food industry, since saturated fats, unlike unsaturated liquid oils, feature a solid consistency and provide the appropriate texture and stability to the finished food. Their exclusion may lead to a decrease in the consumer properties of the food products [6]. The solution to this problem can be found in oleogels and hybrid gels, which are healthy substitutes for solid fats obtained by converting liquid unsaturated oil into an elastic solid product with the help of organogelators and without changing the chemical characteristics of the liquid oil [7,9].

As can be seen, the process of obtaining and applying the various forms of oleogels and hybrid gels has been well studied in recent decades by the foreign scientists and domestic researchers at the scientific school of A. A. Kochetkova [4,13,15]. However, relatively few clinical evaluations of the volunteers who consumed the hybdrid gels, and biomedical studies of impact of oleogels and hybrid gels on the health status of laboratory animals have been conducted. For example, clinical trials of oleogels were organized in 2008 by the scientists from the University of Guelph (Canada) under the leadership of the professor Marangoni [31], who possesses extensive experience in the creation and application of oleogels in food industry. They conducted studies of the lipid composition of the relevant blood fraction in the volunteers after consuming the developed monoglyceride gels. Studies have shown that the blood lipid composition was lower in the group of the volunteers who consumed the developed monoglyceride gel than in the volunteers who consumed a compositionally equivalent oil-water mixture [31].

Later studies conducted by this group of the scientists have shown that coconut oil in the form of oleogel, compared with coconut oil in liquid form, featured better effect on triglycerides, glucose, insulin levels in the blood and appetite when consumed simultaneously with foods rich in carbohydrates [32].

Korean researchers conducted studies to determine the effect of edible oleogel on lipid metabolism in rats fed with a high-fat diet, and found that the use of oleogel based on rapeseed oil and beeswax in the diet effectively reduces adipogenesis and improves angiogenesis in obese rats [33]. Similar studies were conducted earlier by another group of scientists [34], who showed that the use of oleogel reduced the level of triglycerides by about 30 % in blood serum and liver, and increased the level of triglycerides excreted in the faeces by about 30 % compared with rats fed with separate components (rice bran wax and rice bran oil).

In this regard, conducting biomedical research of new forms of edible hybrid gels on laboratory animals remains relevant.

This study is aimed to develop and evaluate the safety and tolerability of edible hybrid gel in an *in vivo* experiment conducted on Wistar rats.

2. Materials and methods

2.1. Materials

The following prescription ingredients were used as material for the preparation of gels: sodium alginate (Parsian Gum Tam Co., Iran), beeswax (Zanburkala Co., Iran), grape seed oil (Monini Co., Italy), drinking water.

2.2. Oleogels and hybrid gels preparation

Oleogel samples were prepared by mixing 60 ml of grape seed oil with 10, 15, and 20 % beeswax according to the procedure presented in the article [29]. To produce a hybrid gel, 10 ml of 2 % sodium alginate in the form of a hydrogel [28] was mixed with oleogel, which contained 20 % beeswax, at four different ratios of hydrogel: oleogel (0:100, 1:99, 5:95, 10:90) [17] by mixing using a mechanical stirrer RZR2102 control (Heidolph, Germany) at 600 rpm for 45 minutes [28].

2.3. Microstructure of oleogels and hybrid gels

For analyze the shape and size of the crystals in oleogels and hybrid gels, transient polarized light microscopy (model BX60, Olympus Optical Co. Ltd, Japan) was used in dark and light fields at magnification of 100 times for oleogels and 400 times for hybrid gels.

2.4. Textural characteristics of oleogels and hybrid gels

The textural characteristics of oleogels and hybrid gels were studied using a texture analyzer (TA.XTplusC, Stable micro system, England). To assess the hardness of each oleogel sample, a penetration test was performed using a 2 mm penetrating probe 50 kg. The texture test was performed at least three times with a pre-penetration rate, a rate during penetration, and a post-penetration rate of 1, 2, and 10 mm/s, respectively, and a penetration depth of 5 mm at a temperature of 25 °C. Exponent software of version 6.1.1.0 was used to analyze the texture tests values.

2.5. Thermal properties of oleogels and hybrid gels

The thermal properties (initial and final melting points) of the samples were evaluated by differential scanning calorimetry (DSC-600 model, Wuhan Bonnin Technology Ltd., China) using SPICO software (DSC1.0.0, China). To conduct the experiment, a sample weighing 18 mg was placed in an aluminum container, hermetically sealed, and heated from 25 °C (T_o is the initial melting point) to 100 °C, (T_m is the maximum melting point) at a heat rising rate of 5 °C/min [28].

2.6. Animal experiments

The experiment with laboratory animals was conducted in the vivarium of the Saratov State University of Genetics, Biotechnology and Engineering named after N. I. Vavilov in accordance with the "Rules for work using experimental animals" (dated 12. Aug. 1977 No. 755), "International principles of the Helsinki Declaration on Humane Treatment of Animals", GOST ISO 10993-1-2011[1]. These studies were conducted according to the approved protocol and in accordance with the researcher's Standard Operating Procedures. The format of the study is defined by the "Rules for conducting a preclinical study of a medicinal product for veterinary use, a clinical study of a medicinal product for veterinary use, a bioequivalence study of a medicinal product for veterinary use" (No. 101 dated 06.Mar. 2018) and the "Guidelines for conducting Preclinical Studies of medicinal Products. Part One (2012). The zoohygienic conditions of all experimental animals were identical, regulated by the recommendations for the maintenance and feeding of laboratory animals (GOST 33215-2014)[2]. According to the study protocol, white Wistar rats with an initial body weight of 180–185 g participated in the experiment. The rats were split into three groups for the study with 12 animals each. The first group of rats (n = 12) was individually and orally fed with the test hybrid gel at a dose of 1 g / kg, once a day, for 30 days, the second group of rats (n = 12) received the hybrid gel orally at a three-fold increased dose of 3 g / kg, according to the same scheme. The third (control) group of rats (n = 12) was fed with a standard diet and did not receive a hybrid gel.

[1] GOST ISO 10993–1–2011. «Medical devices. Biological evaluation of medical devices. Part 1. Evaluation and testing». Retrieved from https://docs.cntd.ru/document/1200100813 Accessed October 12, 2024

[2] GOST 33215–2014 «Guidelines for accommodation and care of animals. Environment, housing and management» Retrieved from https://docs.cntd.ru/document/1200127789 Accessed October 14, 2024

Daily observations of experimental animals were carried out throughout the experiment. At the same time, attention was focused on the appearance and behavior of rats, their consumption of feed and water. The body weight of the animals was determined one day before the start of the experiment and kept being measured weekly until the end of the experiment. Body temperature was measured using a 153-IRB infrared thermometer (Bioseb SAS, France). At the beginning of the experiment, individual body weight values did not deviate from the average value in the group by more than 10%. The animals were weighed on a PA2102C scale (Ohaus, USA).

To assess the effect of the hybrid gel on the hematological and biochemical parameters of animal blood, it was aspirated before the start of the experiment and on the 30th day after the last feeding with the hybrid gel.

Blood was taken from the left ventricle of the heart using an inhalation anesthetic (isoflurane); the skin at the aspiration site was previously disinfected with a 95% solution of ethyl alcohol.

Clinical blood analysis was performed on a MicroCC-20Vet hematology analyzer (High Technology Inc., USA).

Biochemical studies of blood serum (determination of total protein, albumin, globulin, creatinine, urea, glucose, and the activity of major enzymes) were performed on a StatFax 3300 analyzer (Awareness Technology Inc., USA) using diagnostic systems from Deacon DS (Russia).

Histological sections of 5–7 microns thick were cut off using a Microm HM 525 freezing microtome (Microm International GmbH, Germany) and a Microm HM 450 sledging microtome (Microm International GmbH, Germany). For an overview, the prepared histological sections were stained with hematoxylin and eosin. Histological studies were performed using a Biomed C-1 biological microscope (Micromed, Russia) and an LF — 302 fluorescence microscope (Leader Precision Instrument Co. Ltd, China) at a magnification of 100 times. Morphometric data — the radius from the central veins to the wall of the liver lobules — were examined using a biological microscope with an eyepiece ruler of 60 notch divisions and a screw eyepiece micrometer MOB-1–15x (Micromed, Russia).

2.7. Statistical analysis

All studies of oleogels and hybrid gels were carried out three times. The SPSS software (version 16, USA) was used to create a fully randomized complete factor analysis to compare samples with a significance level series at 5%. The version 2010 multiple Duncan test was used to compare averages, and Microsoft Excel was used to plot graphs.

The results of studies on laboratory animals are presented as average values with a significance level of $P=0.05$ compared with the parameters of intact animals. Statistical data processing was carried out on the basis of Fischer and Student criteria.

3. Results and discussions

3.1. Microstructure, textural characteristics and thermal properties of oleogels

The microstructure of oleogels was examined under a polarized light microscope for better understanding the impact of beeswax concentration (10%, 15%, and 20%) on crystal formation (Figure 1).

As can be seen from Figure 1, the crystal network undergoes significant changes along with an increase in the concentration of beeswax in the system. Large number of crystals are observed as the beeswax concentration increases. The smallest crystals were found in an oleogel sample with a beeswax concentration of 10%. Since the oleogel mixture undergoes a cooling process and becomes solid, the increased presence of beeswax molecules in the gel matrix gives the crystals more opportunities to orderly arrange themselves in close proximity to each other. Consequently, this leads to the formation of larger crystal structures [28].

The textural parameters of oleogels from grape seed oil and beeswax were studied at concentrations of 10%, 15% and 20%, as they are shown in Table 1. The results of this study are consistent with the results published by Öğütcü and Yılmaz [10], as well as Hwang et al. [35], which prove that an increase in wax concentration in oil leads to an increase in the hardness and adhesiveness of oleogels.

Table 1. **Textural characteristics of oleogels**
Таблица 1. **Текстурные характеристики олеогелей**

Texture characteristics	Beeswax concentration in the oleogel composition		
	20%	15%	10%
Hardness, N	23.09 ± 0.95^a	5.74 ± 0.12^b	1.92 ± 0.07^c
Adhesiveness, $N.s^{-1}$	-14.45 ± 0.67^a	-14.44 ± 0.81^a	-3.74 ± 0.10^b

a, b, c — average data of repetitions in one line with the same superscripts do not differ significantly ($P>0.05$).

As can be seen from the data, there was a significant difference in the hardness of oleogel samples with concentrations of 20, 15, and 10%. This increase may be due to the higher content of beeswax molecules available in the gel matrix. As more and more of the liquid phase (oil) enters the crystal mesh of the beeswax, the structure of the oleogel solidifies [36].

In a study conducted by Öğütcü and Yılmaz [10], the textural properties of walnut oil oleogels with the addition of beeswax were evaluated, which showed that an increase in the concentration of oleogel in the composition leads to an increase of hardness and adhesiveness. Similarly, another study using soybean oil oleogels with sunflower wax showed similar results, especially with respect to the strength parameter, along with an increase in wax concentration [37, 38].

For further analysis of the characteristics of crystals formed in oleogels, the method of differential scanning calorimetry was applied. The thermal properties of oleogels (initial (T_o) and maximum (T_m) melting points are shown in Table 2.

Table 2. **Thermal properties of oleogels**
Таблица 2. **Термические свойства олеогелей**

Thermal properties	Beeswax concentration in the oleogel composition		
	20%	15%	10%
T_o, °C	56.7 ± 0.46^a	55.1 ± 0.61^b	52.0 ± 0.53^c
T_m, °C	59.8 ± 0.70^a	58.2 ± 0.49^b	56.0 ± 0.38^c

a, b, c — average data of repetitions in one line with the same superscripts do not differ significantly ($P>0.05$).

In a study conducted by Jana and Martini [39], it was found that certain processing conditions can influence on phase separation in wax/oil systems. The wax concentration and the type of oil are important factors influencing the wax crystallization process. An increase in the beeswax concentration in the oleogel leads to an increase in the number of beeswax molecules in the system, which leads to a more uniform appearance of the final product. This is consistent with the conclusions of the other researchers [10]. The initial and maximum melting points increased along with an increase in the percentage of beeswax in the oleogels.

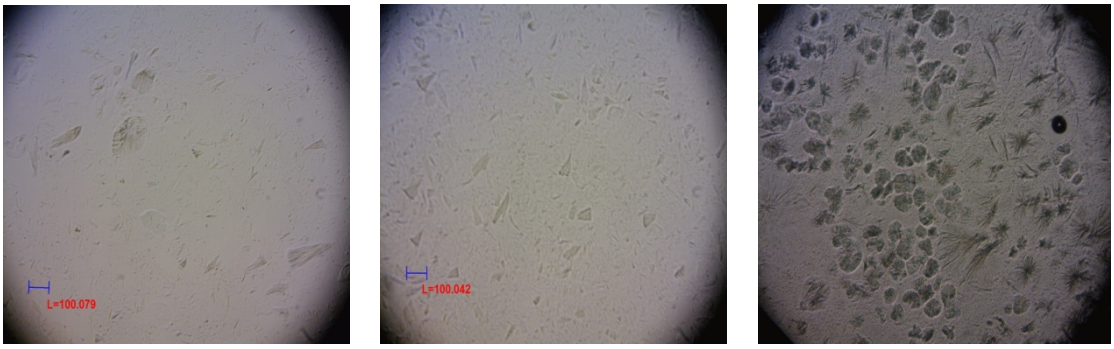

Figure 1. **Microstructure of oleogels (A – oleogel with 10% beeswax, B — oleogel with 15% beeswax, C — oleogel with 20% beeswax)**
Рисунок 1. **Микроструктура олеогелей (А — олеогель с 10% пчелиного воска, В — олеогель с 15% пчелиного воска, С — олеогель с 20% пчелиного воска)**

This phenomenon can be explained by an increase in the number of crystals formed due to an increase in the surface area of beeswax molecules in the mixture. As the number of crystals increases, the temperature of the crystallization beginning and the melting point also increase.

In their study, the scientists [27] used a combination of walnut oil with beeswax and monoglycerides. The results showed that the melting point and enthalpy of oleogel were generally similar in the samples of commercial shortening. Thus, these oleogels can be considered suitable alternatives for similar applications and do not create problems with the thermal regime of food products processing. In addition, an increase in the concentration of oleogel led to an increase in the melting point and enthalpy values in the samples. This discovery points to an important technological aspect in which the appropriate level of oleogel addition can be determined based on thermal parameters and other measurements, taking into account specific end-use requirements (for example, food texture, taste, etc.).

Thus, based on the research conducted, an oleogel sample with a beeswax concentration of 20% was selected for the production of hybrid gels, as it demonstrated the best textural characteristics and thermal properties. The developed sample possessed the taste and smell of beeswax, but this effect can be masked with flavoring agents when developing food products.

3.2. Microstructure, textural characteristics and thermal properties of hybrid gels

The microstructure of hybrid gels was studied using a polarized light microscope. The data obtained show that as the hydrogel ratio in the hybrid gel increased, the average particle size also increased slightly, as shown in Figure 2.

However, no significant differences were found ($P>0.05$), possibly due to the fact that all hybrid gel samples were produced using the same mixing process (mixing speed), resulting in particles of approximately identical size. This conclusion is consistent with the research conducted by Martins and co-authors [8].

When making hybrid gels, it is important to take into account the ratio of the gel-forming phases (hydrogel and oleogel) in the overall gel composition. Table 3 shows the hardness and adhesiveness data for various hybrid gel samples.

Table 3. **Textural characteristics of hybrid gels**

Таблица 3. **Текстурные характеристики гибридных гелей**

Texture characteristics	Hydrogel: oleogel ratio in hybrid gels			
	0:100	1:99	5:95	10:90
Hardness, gf	321.47±3.60^{c}	323.37±4.22^{c}	396.13±4.58^{b}	472.27±3.67^{a}
Adhesiveness, gf.s^{-1}	154.40±4.21^{c}	143.07±3.86^{d}	187.23±4.32^{b}	242.23±4.32^{a}

a, b, c — average data of repetitions in one line with the same superscripts do not differ significantly ($P>0.05$).

It can be seen from the data in Table 3 that the textural characteristics of hybrid gels increase along with an increase in the hydrogel ratio in the hybrid gel, which is most likely due to an increase in the number of hydrogen bonds. The greater the number of hydrogen bonds, the stronger the sample becomes. The hardness data did not show a significant difference between the 1:99 sample and the control sample, as the ratio was not high enough to set sufficient hydrogen bonds. However, significant differences were already observed in the hydrogel: oleogel ratio of 5:95 and 10:90, respectively [38].

Figure 3 shows data on the thermal properties of the hybrid gels under study.

As can be seen from the data in Figure 3, the initial and maximum temperatures of the hybrid gel samples melting increased along with an increase in the amount of hydrogel in the hybrid gel, which can also be explained by the high moisture content in the hybrid gel system, leading to the formation of more hydrogen bonds. Their destruction requires more energy, which leads to an increase in the system melting point temperature along with increasing the share of hydrogel in the hybrid gel.

Previous researchers [39] reported that hydrocolloidal molecules in the form of intramolecular and/or intermolecular bonds contribute to the formation of denser layers in oleogels. It takes more energy to overcome these bonds. In addition, the beeswax in the hybrid gel system forms a gel-like mesh that traps oil droplets and potentially binds to hydrocolloid molecules. This mesh creates a dense environment and is able to trap water molecules. The peak values for hybrid gels of 10:90 and 5:95 showed no significant difference ($P>0.05$). This indicates that in the 5:95 sample, almost all vegetable oil molecules formed bonds with trapped water molecules, while in the 10:90 sample the number of bonds did not increase. This fact is also confirmed by the research of Kwon and Chang, demonstrating that the excessive water in the gel system was retained inside the oil droplets by beeswax, without increasing the number of intermolecular bonds between water and vegetable oil [40].

One sample of a hybrid gel in a hydrogel: oleogel ratio of 5:95 was selected as the best one to study safety and tolerability.

3.3. Assessment of safety and tolerability of edible hybrid gel oral administration in an experiment on Wistar rats

In the course of the conducted studies, it has been reliably established that prolonged oral administration of the hybrid gel for 30 days does not adversely affect the basic physiological parameters of the rats. The experimental animals were active throughout the experiment, reacted adequately to external stimuli, and actively consumed food and water. The appearance of the animals corresponded to the species criteria, no grooming violations were noted. No side effects, adverse reactions, or serious adverse reactions were detected in laboratory animals during and after the use of the hybrid gel preparation in the recommended and three-fold increased dose. Table 4 shows data on the rats body temperature dynamics in the course of their consuming hybrid gel. As can be seen throughout the experiment, the parameters remained within the limits of normal physiological values in all groups.

Table 4. **Dynamics of body temperature in rats after oral administration of hybrid gel**

Таблица 4. **Динамика температуры тела крыс при применении гибридного геля**

Name of the group	Body temperature in rats during the experiment, °C	
	1st day	30th day
First group (experimental group)	40.9±0.5^{a}	40.9±0.4^{a}
Second group (experimental group)	41.0±0.3^{a}	40.6±0.4^{a}
Third group (control group)	40.8±0.5^{a}	40.6±0.3^{a}

^{a} — average data of repetitions in one line with the same superscripts do not differ significantly ($P>0.05$).

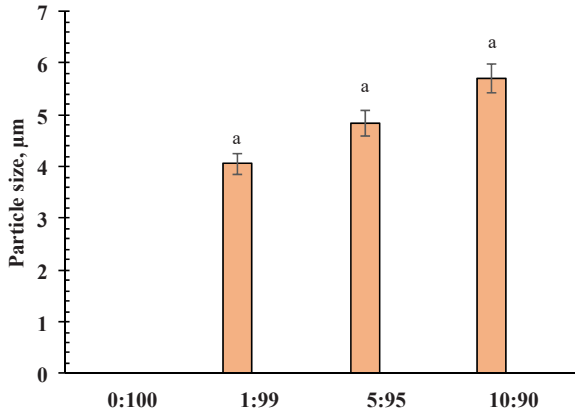

Figure 2. **Particle size of hybrid gels**
(a — measurement error according to the statistical analysis data)

Рисунок 2. **Размер частиц в образцах гибридных гелей**
(a — погрешность в соответствии с данными по статистическому анализу)

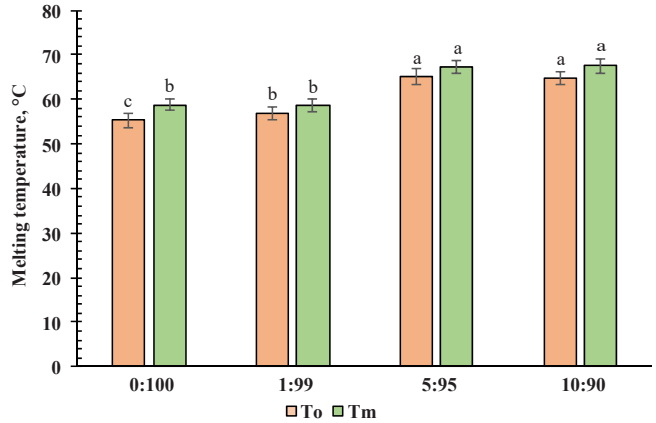

Figure 3. **Thermal properties of hybrid gels**
(a, b, c — measurement errors according to statistical analysis data)

Рисунок 3. **Термические свойства гибридных гелей**
(a, b, c — погрешности в соответствии с данными по статистическому анализу)

Table 5 shows the hematological parameters of rat blood after oral administration of the hybrid gel, which proves that before the start of the experiment hematological parameters of peripheral blood in all groups of rats were within the normal physiological values. In animals of the first and second experimental groups, on the first day after the last oral administration of the hybrid gel, no significant differences were found between the final and initial values. From the results obtained, it can be concluded that the use of the developed hybrid gel in the diet of Wistar rats in a single and three-fold increased dose for 30 days does not provide a toxic effect on the hematological parameters of animal blood.

Table 5. **Hematological parameters of rat blood after oral administration of hybrid gel**

Таблица 5. **Гематологические показатели крови крыс после перорального введения гибридного геля**

Parameter	Days of the experiment	Name of the group: First group (experimental group)	Second group (experimental group)	Third group (control group)	Norm
Erythrocytes, $\times 10^{12}$/L	0	7.25 ± 0.7^a	7.29 ± 0.4^a	7.14 ± 0.5^a	4.6–10
	30	7.38±0.7^a	7.68 ± 0.7^a	7.29 ± 0.6^a	
Hemoglobin, g/L	0	117.9 ± 6.2^a	120.4 ± 5.7^b	115.7 ± 8.3^a	93–153
	30	125.3 ± 6.4^a	119.3 ± 6.4^b	126.5±5.4^a	
Leukocytes, $\times 10^9$/L	0	10.8 ± 2.6^a	12.7 ± 3.4^b	11.9 ± 4.9^c	5.5–19.5
	30	4.8 ± 3.9^a	8.9 ± 1.5^b	12.4 ± 3.6^c	
Neutrophils, %	0	26.2 ± 1.8^a	27.6 ± 2.7^a	26.2 ± 3.2^a	18–30
Eosinophils, %		5.6 ± 0.7^a	6.2 ± 0.6^b	5.2 ± 1.6^a	1–8
Monocytes, %		3.8 ± 1.0^a	4.0 ± 1.2^b	4.2 ± 1.0^b	0–10
Basophils, %		0.0 ± 0.0^a	0.4 ± 0.7^b	0.6 ± 0.7^c	0–3
Lymphocytes, %		24.3 ± 1.9^a	23.6 ± 3.9^a	24.8 ± 3.2^a	12–45
Neutrophils, %	30	26.0 ± 3.4^a	25.2 ± 3.2^a	26.6 ± 2.6^a	18–30
Eosinophils, %		4.8 ± 1.4^a	4.8 ± 1.4^a	5.2 ± 1.6^b	1–8
Monocytes, %		3.8 ± 1.0^a	3.6 ± 1.1^a	4.6 ± 0.7^b	1–10
Basophils, %		0.2 ± 0.6^a	0.4 ± 0.7^b	0.5 ± 0.7^c	0–3
Lymphocytes, %		22.8 ± 3.0^a	66.2 ± 3.0^b	63.6 ± 3.4^b	12–45

a, b, c — average data of repetitions in one line with the same superscripts do not differ significantly (P>0.05).

When analyzing the biochemical parameters of the blood serum of the rats (Table 6), which were orally injected with an edible hybrid gel daily for 30 days, it was reliably established that there was no negative effect on the hepatocellular and urinary systems. Given the fact that the liver is the central organ of metabolism and it plays a major role in the detoxification of nutrients supplied with the feed, the negative impact of enteral food products will primarily affect liver's functional activity. During the experiment on the oral administration of the hybrid gel, no violations of the functional activity of hepatocytes were detected. Along with this, the kidneys are responsible for removing metabolic products from the animal's body. Thus, it can be stated that the metabolites of the edible gel do not adversely affect the functional activity of the urinary system. These statements are confirmed by the absence of changes in biochemical parameters in all experimental groups of the rats throughout the experiment.

Another parameter that highlights the physiological development of the rats is body weight increase. The results of the dynamics of the rats body weight increase against the background of edible hybrid gel consumption for 30 days are shown in Table 7.

Analyzing the data, it was found that the weight growth rates of the rats that consumed the edible hybrid gel and the rats in the control group had no significant differences, and all of them corresponded to the normal weight criteria for this rat breed. The weight increase in 30 days was 39 ± 2 g for the first experimental group, 38 ± 2 g for the second experimental group, and 41 ± 1 g for the third control group.

The results of the conducted studies allow concluding that after a course of oral administration of the edible hybrid gel, no external signs of intoxication were observed in the rats throughout the experiment. All the rats in both the experimental and control groups were active. The reaction to external stimuli was preserved, appetite and water consumption were not impaired. The dynamics of body weight gain in the rats of the experimental groups had no significant differences between the control group, and corresponded to the breed criteria.

Table 6. **Biochemical parameters of rat blood serum after oral administration of hybrid gel**

Таблица 6. **Биохимические показатели сыворотки крови крыс после перорального введения гибридного геля**

Parameter	Days of the experiment	Name of the group: First group (experimental group)	Second group (experimental group)	Third group (control group)	Norm
AST, IU/L	0	206.8 ± 2.2^a	208.3 ± 1.9^c	207.9 ± 3.2^b	60–223
	30	138.9 ± 2.3^a	173.7 ± 2.7^b	209.9 ± 2.7^c	
ALT, IU/L	0	59.9± 3.3^a	63.7± 2.3^b	64.9± 1.3^c	34–76
	30	38.5± 1.3^a	61.1± 3.4^b	65.5± 1.5^c	
Creatinine, mmol/L	0	65.8 ± 2.5^a	67.3 ± 4.7^c	63.4 ± 5.0^b	44–85
	30	83.7 ± 1.3^c	74.4 ± 3.1^b	62.1 ± 6.2^a	
Urea, mmol/L	0	5.7 ± 3.1^a	6.9 ± 2.1^c	5.9 ± 1.1^b	3–7.8
	30	6.9 ± 1.1^c	6.3 ± 2.3^b	5.4 ± 1.3^a	
Glucose, mmol/L	0	12.2 ± 1.6^a	12.8 ± 2.2^c	12.4 ± 2.1^b	3–13
	30	13.1 ± 1.9^c	12.8 ± 1.2^b	12.4 ± 1.8^a	
Albumin, g/L	0	24.8 ± 1.9^b	25.3 ± 2.8^c	24.1 ± 1.7^a	25–38
	30	25.8 ± 1.5^b	26.1 ± 2.8^c	23.7 ± 1.8^a	
Total protein, g/L	0	53.8 ± 3.6^b	54.1 ± 3.2^c	51.6 ± 1.2^a	59–82
	30	54.6 ± 4.3^b	54.9 ± 3.3^c	52.1 ± 2.8^a	
Globulin, g/L	0	29.0 ± 3.2^b	28.8 ± 3.1^b	27.5 ± 1.8^a	20–32
	30	28.9 ± 4.2^a	28.8 ± 4.2^a	28.4 ± 2.2^a	
Alkaline phosphatase, mmol/L	0	2.6 ± 0.7^a	3.1 ± 1.6^b	2.9 ± 0.6^a	2–4
	30	3.2 ± 0.9^b	2.6 ± 0.8^a	2.7 ± 1.1^a	

a, b, c — average data of repetitions in one line with the same superscripts do not differ significantly (P>0.05).

Table 7. **Dynamics of body weight gain in the rats after oral administration of hybrid gel**

Таблица 7. **Динамика прироста массы тела у крыс после перорального введения гибридного геля**

Days of the experiment	Weight of rats after oral hybrid gel administration, g: First group (experimental group)	Second group (experimental group)	Third group (control group)
1st day	180 ± 7^a	181 ± 5^a	185 ± 6^a
30th day	219 ± 4^a	219 ± 3^a	226 ± 3^b

a, b — average data of repetitions with the same superscripts do not differ significantly (P>0.05).

The biochemical parameters of blood corresponded to physiological norms, while there were no significant changes in those values in the experimental and control groups during the experiment. The data obtained proves the absence of violations of the functional state of the kidneys and liver in experimental laboratory animals.

On the 30th day of the experiment, one animal was selected from each group, and euthanasia was performed with subsequent liver extraction for histological studies (Figure 4).

Analyzing the data in Figure 4, it can be seen that a clear, multifaceted lobular liver parenchyma is visualized on histological sections, delimited by loose connective tissue. Inside the lobules, hepatocyte cells are quite expressed in the form of a hexagon, the nuclei of the latter are sufficiently visualized in the amount of 2–3 pieces. Between the lobules, the liver triads are clearly visible, represented by the interlobular veins and arteries, as well as the bile duct. In the liver of laboratory animals of all experimental groups, the walls of the central vessels of the lobules are sufficiently structured, their layers are well expressed, a small number of them were blood-filled, which indicates a more intensive blood circulation in the largest wall-mounted digestive gland.

The morphometric parameters of the liver (radius from the lobule wall to the central vein) of all experimental groups of rats are shown in Table 8.

Table 8. **Morphometric parameters of the liver**

Таблица 8. **Морфометрические показатели печени**

Radius of liver lobules, microns		
First group (experimental group)	Second group (experimental group)	Third group (control group)
11.21 ± 0.53^b	13.42 ± 0.52^c	08.10 ± 0.41^a

a, b, c — average data of repetitions in one line with the same superscripts do not differ significantly (P>0.05).

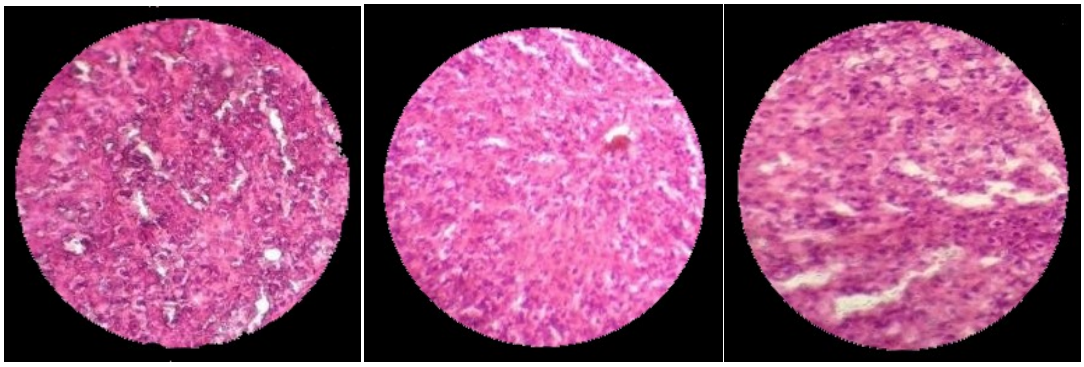

Figure 4. **Histological studies of rats liver after oral administration of hybrid gel** (A — first experimental group, B — second experimental group, C — control group). Staining with hematoxylin and eosin, at magnification ×100

Рисунок 4. **Гистологические исследования печени крыс после перорального введения гибридного геля** (А — первая экспериментальная группа, В — вторая экспериментальная группа, С — контрольная группа). Окраска гематоксилин и эозином, при увеличении ×100

Table 8 shows that the radius from the central vein to the lobule wall in the rats of the control group averaged 08.10±0.41 microns, in the first experimental group — 11.21±0.53 microns, and in the second — 13.42±0.52 microns.

Data analyses from histological and morphometric studies of the liver of the rats from the control group and experimental group demonstrated that no significant differences were detected, which proves the safety of using the hybrid gel in the diet of the animals.

4. Conclusions

Analysis of the microstructural, textural and thermal properties of oleogels and hybrid gels has shown that beeswax improves textural and thermal characteristics, while the hydrogel portion affects the adhesion and hardness of gel matrices. During the research it was reliably established that prolonged oral administration of the hybrid gel did not cause clinical signs of intoxication, the rats of all experimental groups adequately reacted to external irritating factors throughout the study period, the rats were active, their appetite and water consumption were relevant to their natural physiological form. During the experiment, a positive increase in body weight was observed, which corresponded to the age and physiological norm of the laboratory rats. Based on the results obtained, the safety and tolerability of hybrid gel oral administration in Wistar rats was proved. The prospects for creating edible hybrid gels seem promising, as they solve current dietary and health issues while providing functional and sensory benefits in food formulations. Continuous research and progress in this area will be crucial to unlock the entire potential of these innovative ingredients in the food industry.

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Conflict of interest	**Конфликт интересов**
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