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Research Article

Preclinical safety evaluation of drone brood homogenate and justification of pharmacological action

Bohdana Pavliuk[1], Iryna Stechyshyn[1], Mariana Chubka[1], Taras Hroshovyi[1]

1 *I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine*

Corresponding author: Bohdana Pavliuk (vons@tdmu.edu.ua)

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Abstract

The problem of studying the metabolic syndrome, as well as its integration into other pathological processes, despite large-scale research, remains relevant. The complexity of the interaction of different links in pathogenesis requires scientists to find new tools and methods for both diagnosis and treatment. Drone brood homogenate, which is a multifactorial pharmacological agent in terms of chemical composition, seems to be promising to study for today. And the lack of contraindications and a wide age range makes it an excellent object of research. The current study evaluated the pharmacological aspects of safety: acute toxicity, effects on the functional and motor activity of the gastrointestinal tract, as well as local irritation of the gastric mucosa, the secretory function of the stomach. All experiments were performed according to the classical methods. The specific pharmacological activity of the drone brood homogenate was determined in comparison with metformin in the experimental fructose metabolic syndrome. Animals obtained from the Vivarium of I.Horbachevsky Ternopil National Medical University were used to implement the set goals. Working with animals was met all bioethical requirements. The study found that the lyophilized drone brood homogenate does not have a local irritant effect and does not cause ulcers on the surface of the gastric mucosa, does not affect the secretory function of the stomach and motor-evacuatory activity of the gastrointestinal tract and is a low-toxic substance, indicating the possibility of its long-term safe use. As expected, glucose, insulin, and HOMA index were significantly increased in animals that were simulated metabolic syndrome. The use of drone brood homogenate by animals contributed to a relatively positive effect on selected indicators of the metabolic syndrome. Accordingly, drone brood homogenate is a promising active pharmaceutical ingredient for the normalization of biochemical disorders in metabolic syndrome.

Keywords

metabolic syndrome, fructose, glucose, insulin, drone brood homogenate

Introduction

The metabolic syndrome (MetS) is one of the priority and socially significant problems of modern medicine, which attracts the attention of a wide range of specialists from around the world – cardiologists, endocrinologists, therapists, geneticists, general practitioners, etc. (Pavliuk et al. 2020; Kritsak et al. 2021). MetS is an integral symptom complex of combined pathological processes, including diabetes mellitus and cardiovascular diseases (Pavliuk et al. 2020; Stechyshyn et al. 2020; Pavlyshyn et al. 2021).

The relationship described in Figure 1 shows that MetS is a set of metabolic disorders that potentiate each other, forming a pathologically connected circle that is

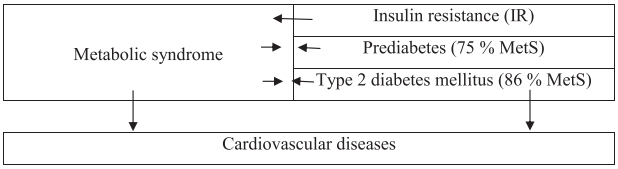

Figure 1. Relationships between metabolic syndrome, insulin resistance, pre-diabetes and type 2 diabetes mellitus.

very difficult to break (Stechyshyn et al. 2020; Marushchak et al. 2021).

It is generally believed that the formation of MetS occurs with the direct participation of insulin resistance (IR) which triggers a cascade of metabolic disorders, leads to severe cardiovascular complications and has therefore been identified as an independent risk factor for cardiovascular disease (Roberts et al. 2013; Jinkyung Cho et al. 2017; Gluvic et al. 2017). According to the literature, the basis of IR syndrome is a decrease in insulin sensitivity in combination with concomitant hyperinsulinemia and atherogenic dyslipidemia. It has been studied that there is a clear relationship between the most important components of the IR-syndrome; the greater the degree of reduced insulin sensitivity, the higher it is content and the risk of developing disorders associated with hyperinsulinemia. On the other hand, the greater the severity and range of disorders, both metabolic and functional, the higher the risk of IR (Roberts et al. 2013; Gluvic et al. 2017). The search for new drugs that could reduce the negative effects of MetS on the body, speed up regeneration processes and be as harmless as possible, seems obvious and relevant in this case. In this regard, promising is the study of bee`s products, in particular, drone brood homogenate (DBH); which is known to increase metabolism during the active muscular activity of the body, affecting the growth of physical performance, able to stimulate the immune`s systems (the production of antibodies by the spleen and the immune response of T-lymphocytes), and reduce oxidative stress and risk of death from cardiovascular disease (Sawczuk et al. 2019; Sidor et al. 2020). The high natural biological activity of DBH is due to a unique combination of components with certain physiological properties: hormone-like effect, rejuvenating effect, normalization of blood pressure, lowering cholesterol, effects on metabolism, and others (Sawczuk et al. 2019; Sidor et al. 2020). In addition, the lack of contraindications and a wide age range make it a great object for study (Sidor et al. 2020).

The chemical composition of DBH is characterized by the presence of proteins, amino acids, nucleic acids, enzymes, phospholipids. Also, a complex of substances of the lipid fraction, mono-, di- and hydroxycarboxylic acids, fatty acids, steroid hormones, carbohydrates, flavonoids, a wide range of micro- and macronutrients, water- and fat-soluble vitamins A, D, E, PP, C, group B and many other biologically active components, which is known from the literature (Izuta et al. 2009; Karomatov 2020; Sidor et al. 2020).

The presence in the composition of the DBH of substances with a wide range of pharmacological action makes it possible to influence many parts of the metabolic syndrome. In particular, the presence of phytosterols, which do not cause hormonal disorders and contribute to the stimulating effect on the endocrine system in general (Sawczuk et al. 2019) and, most importantly, dyslipoproteinemia, which plays a key role in the progression of metabolic syndrome. Due to the similarity of their structure to cholesterol, phytosterols bind to low-density lipoproteins, preventing the formation of a more atherogenic compound. Phytosterols, when regularly ingested, can lower the cholesterol of low-density lipoproteins and total cholesterol by an average of 10–15% (Ito et al. 2011; FDA 2008; Casas-Agustench et al. 2012; Maki et al. 2012). Long-term studies lasting up to 85 weeks have shown that the effect of lowering cholesterol can be sustainable (EFSA 2009).

The presence of phytosterols in the DBH provides anabolic activity, which is enhanced by the presence of amino acids, trace elements, vitamins A and B. The presence of B vitamins in the drone homogenate also positively promotes the metabolism of proteins, fats, hydrocarbons. And also for the synthesis of acetylcholine – the main neurohumoral mediator that balances the production of NO (Koh et al. 2009; Chis et al. 2015). It is known that the violation of NO synthesis is one of the manifestations of endothelial dysfunction, which is an independent factor influencing the development and progression of microvascular pathology.

Among the above substances present in the DBH, of great value is 10-oxo-2-decenoic acid (Izuta et al. 2009). By binding excess peroxide compounds in metabolic syndrome, drone homogenate directly affects another important factor, oxidative stress (Pavliuk et al. 2020; Marushchak et al. 2021). Nowadays, as the basis of the universal theory of the development of all complications in MetS, oxidative stress is considered, including as a result of endothelial dysfunction and depletion of antioxidant protection (Stechyshyn et al. 2020). Moreover, oxidative stress induced by hyperglycemia triggers the mechanisms of β-cell damage. Due to the presence of flavonoids, the DBH also has an anti-inflammatory effect, enhancing the antioxidant effect (Karomatov 2020).

Drone homogenate increases the level of metabolism during active muscular activity, which increases physical endurance (Ahmad et al. 2020; Ghosh et al. 2020).

Materials and methods

Before the study of specific pharmacological action according to the guidelines of the State Export Center (SEC) of the Ministry of Health of Ukraine (MHU) "Preclinical safety evaluation of biotechnology-derived pharmaceuticals" is recommended to study the pharmacology of safety of the active pharmaceutical ingredient (APhI), namely, lyophilized DBH powder to establish

the possibility of unexpected pharmacological action, and if necessary, to carry out detailed monitoring of such effects during toxicological studies. The study of safety pharmacology involves the determination of functional indicators of potential toxicity, which contribute to the establishment of mechanisms of specific organ toxicity of the study drug and should be taken into account with respect to human use or indications for use (Stefanov 2001; Slobodianiuk et al. 2020).

In view of this, it was decided to initiate a single-dose toxicity study to obtain information on the relationship between dose and systemic and/or local toxicity. Acute toxicity of the lyophilized DBH powder was studied in accordance with the recommendations of the SEC of the MHU (Stefanov 2001) on white Wistar rats of both sexes (body weight 180–220 g). Lyophilized DBH powder (LLC "Natural Beauty", Ukraine) was administered once in a water solution at a maximum dose of toxicity class IV "Slightly toxic" – 5000 mg/kg. The animals were kept in a vivarium on a standard diet with free access to water. During the experiment (14 days) observed the general condition and behavior of experimental animals. The degree of toxicity of the test agent was determined according to the Hodge and Sterner toxicity scale (Hodge et al. 1943).

For all drugs used in the mode of repeated use, it is necessary to conduct studies of local irritation. Therefore, for a potential oral drug, it was advisable to study its possible effect on the functional and motor activity of the gastrointestinal tract (GIT), as well as the local irritant effect on the gastric mucosa (GM). The study of the possible ulcerogenic effect of the lyophilized DBH on the gastric mucosa was performed on white Wistar rats of both sexes, weighing 200–220 g (Marazzi-Uberti et al. 1961; Fulga et al. 2020). The animals were kept on a starvation diet for 24 hours without water restriction, then lyophilized DBH (LLC "Natural Beauty", Ukraine) was administered intragastrically at doses of 72 mg/kg and the reference drug was acetylsalicylic acid (ASA) (Bayer Bitterfeld GmbH.) at a dose of 100 mg/kg. After 4 hours under sodium thiopental anesthesia, the animals were removed from the experiment, examined the mucous membrane of the stomach of animals using a magnifying glass. The degree of damages to the GIT was evaluated in points: 0-points – no visible damage, 1 point – from 1 to 3 small ulcers, 2 points – more than 3 small ulcers, 3 points – a significant ulcer and several small ulcers, 4 points – several large ulcers, 5 points – perforated ulcer with bleeding. In addition, the symptoms that precede the formation of destruction in the stomach and indicate certain trophic disorders of the mucosa (edema, redness, vascular injection, hemorrhage) were estimated at 0.5 points. Determined the % of animals with lesions of the gastric and intestinal mucosa.

Ulcer index (UI) was calculated by the formula (Mehanna et al. 2020):

$$UI = (\text{degree of ulcer} * \text{percentage of animals with ulcers})/100.$$

In order to study the effect of lyophilized DBH on the functional state of the gastrointestinal tract in rats weighing 180–220 g investigated the secretory function of the stomach according to the method Andreeva A.I. and Sharova S.A. (Andreeva et al. 1978). White rats were prepared for the experiment by keeping them on a starvation diet for 24 hours. One hour after intragastric administration of lyophilized DBH in a conditional therapeutic dose of 72 mg/kg of experimental animals and control animals was anesthetized, and then were ligated to the pyloric sphincter of the stomach, and after 4 hours – to the cardiac. In the animals removed from the experiment, the volume of gastric juice was measured. The intensity of secretion of gastric juice was calculated per 100 g of body weight of the animal. Total and free acidity was determined by titration of gastric juice with 0.1 M NaOH solution in the presence of phenolphthalein and bromothymol blue indicators. Total and free acidity was expressed in titrimetric units: the quantity of 0.1 M NaOH solution in mL which is required for neutralization of 100 mL of gastric juice. Bound acidity was determined by the difference between total and free acidity.

The study of the effect of the lyophilized DBH on the motor-evacuator activity of the GIT was performed by the method of Sticknay J.S. et al (Sticknay et al. 1951) on white mice weighing 21–23 g, which were kept on a starvation diet without the restriction of water intake during the day. Experimental animals were intragastrically administered – lyophilized DBH in a conditionally therapeutic dose, and the intact group – the same amount of water. After 1 hour, all animals were injected intragastrically with 0.3 mL of contrast medium (10% suspension of activated carbon in 1% starch paste). After 40 minutes under thiopental anesthesia, the animals were removed from the experiment. Then in the experimental and control groups of animals measured the absolute length of the intestine and the path traveled by the contrast mass through the intestine in centimeters. As an integral indicator that characterizes the strength of peristalsis of the GIT, using the percentage of intestinal length, passed the contrast mass relative to the absolute length of the entire intestine.

The scope and direction of the study of pharmacological activity of lyophilized DBH powder, according to the SEC of the MHU, was determined depending on the amount of existing scientific information about it (Stefanov 2001). Therefore, based on the known data about the APhI, we choose to investigate its effect under the condition of modeling the MetS.

The study was performed on adult male Wistar rats weighing 230–260 g, which were randomly divided into 5 groups (n = 6): 1 – control, 2 – control pathology (CP), 3 – correction of the drug "Metformin" (SANDOZ, 500 mg, LEK, Poland) at a dose of 60 mg/kg of animal weight, 4 – correction of lyophilized DBH powder (LLC "Natural Beauty", Ukraine) at a dose of 72 mg/kg of animal weight, 5 – correction of lyophilized DBH powder (LLC "Natural Beauty", Ukraine) at a dose of 72 mg/kg of animal weight. Groups 2, 3, 4 instead of water were given a 20% solution of fructose – 8 weeks (Meirelles et al. 2011; Kantar et al. 2015).

Therefore, we studied the effects on insulin transmission in target tissues of a 2-months period of fructose supplementation, comparable to 6 human years of daily fructose consumption (Baena et al. 2016). It is believed that the use of such concentration, in contrast to high-fructose (60–70%), more closely corresponds to the picture of IR in humans and is sufficient for the manifestation of major metabolic disorders in experimental animals (Wong et al. 2016). Animals of group 3 – was the correction of the drug "Metformin" and group 4 – was the correction of lyophilized DBH powder (LLC "Natural Beauty", Ukraine) on the background of fructose load, starting from 6 weeks of MetS modeling, began to be administered intragastrically with a probe in the form of a water solution of metformin for 14 days (therapeutic and prophylactic mode of administration) and lyophilized DBH powder (groups 4), respectively (Barthem et al. 2019; Mengsiyu et al. 2019).

All animals were kept on a standard I. Horbachevsky Ternopil National Medical University vivarium diet, kept under standard conditions at room temperature in isolated cages with a 12-hour day/night, with access to water and food ad libitum. The studies were carried out in accordance with national and international recommendations for the protection of animals used for experimental and other scientific purposes (Strasbourg 1986; Law of Ukraine № 3447-IV 2006)

Fasting glucose was determined using FreeStyle Optium. The degree of sensitivity of the liver and peripheral tissues of animals to the action of insulin was evaluated in a short insulin test. The Homeostasis Model Assessment (HOMA) mathematical model for insulin glucose binding was used to assess insulin resistance.

Calculation of insulin resistance

Insulin resistance was determined using the homeostasis model assessment index for insulin resistance (HOMA-IR) using the following formula:

HOMA-IR index = [fasting glucose (mmol/L) × fasting insulin (μU/ml)]/22.5 as described by Matthews et al. (1985).

The obtained results were subjected to statistical analysis by methods of variation statistics. For all studies, the arithmetic means, as well as the standard error, were calculated. For statistical analysis of the obtained results, a one-way analysis of variance ANOVA was performed using multiple comparative Tukey tests. Statistical analyzes were performed using GraphPad Prism, version 5.0 (GraphPad Software, Inc.).

Results and discussion

A preclinical study of the safety pharmacology of lyophilized DBH powder was performed in accordance with the principles of Good Laboratory Practice. The investigated lyophilized DBH powder was administered as a water solution once intragastrically at a maximum dose of toxicity class IV – 5000 mg/kg. After the introduction of lyophilized DBH powder, no signs of intoxication and manifestations of physiological disorders in rats have observed: the animals were active with a healthy appetite, urination, and defecation identical to the animals of the control group, with neat appearance, etc. Reflex excitability in all animals was preserved. At the end of the observation, the number of animals was unchangeable (n = 6), both in the control group and in the study group.

After removing the animals from the experiment, as a result of macroscopic examination, it was noted that vital organs and systems whose functions are acutely critical for life were unchanged. Therefore, according to the SEC of the MHU and according to the Hodge and Sterner toxicity scale (1943), the obtained results allow carrying the lyophilized DBH powder to the IV class of toxicity – "Slightly toxic".

During a macroscopic examination of the gastric mucosa and duodenum in rats, it was found that lyophilized DBH in all tested doses did not cause damage to the gastrointestinal mucosa, unlike animals injected with acetylsalicylic acid (Table 1).

Against the background of the introduction of acetylsalicylic acid at a dose of 100 mg/kg, it was found that the lyophilized DBH has no ulcerogenic effect, which indicates the possibility of its safe use as a potential oral drug (Table 1).

Table 1. The effect of lyophilized DBH on the condition of the gastric mucosa and duodenum in rats, M ± m (n = 6).

Groups of animals	Degree of damage	Ulcer index
Lyophilized DBH, 72 mg	0	0
Acetylsalicylic acid, 100 mg	6.42 ± 0.14 p ≤ 0.001	4.68 ± 0.12 p ≤ 0.001

As a result of studying the effect of drone homogenate on the secretory function of the stomach, it was found that a conditional therapeutic dose of 72 mg/kg of the studied APhI did not change the volume of gastric contents, free, total, and bound acids (Table 2), indicating the absence of the effect of lyophilized DBH on the secretory function of the stomach.

Table 2. The effect of lyophilized DBH on the secretory function of the stomach of rats, M ± m (n = 6).

Groups of animals	Secretion of gastric juice, ml/100 g of animal weight	Total acidity, ml 0.1 N NaOH/100 ml of gastric juice	Free acidity, ml of 0.1 N NaOH/100 ml of gastric juice	Bound acidity, ml
Control group	2.55 ± 0.17	96.22 ± 0.34	85.23 ± 0.94	10.90 ± 0.46
Lyophilized DBH	2.70 ± 0.14	93.90 ± 1.41 p ≥ 0.05	89.35 ± 0.98 p ≥ 0.05	11.06 ± 0.64 p ≥ 0.05

One of the tasks of studying the pharmacological properties of drugs intended for oral administration is to study their possible effect on the motor-evacuation activity of the GIT. The results of the experiment showed that lyophilized DBH at a dose of 72 mg/kg does not affect the advancement

of the coal suspension in the intestine (Table 3). Therefore, it was found that the strength of intestinal peristalsis in animals under the action of drone brood homogenate compared with the control group does not change.

Table 3. Influence of the lyophilized DBH on motor function of the gastrointestinal tract, M ± m (n = 6).

Groups of animals	Li	Lci	Lci ×100%/Li
Control group	60.32 ± 1.11	42.80 ± 3.05	70.95 ± 2.85
Drone brood homogenate	62.80 ± 1.85	44.05 ± 1.39	70.14 ± 1.16
	p ≥ 0.05	p ≥ 0.05	p ≥ 0.05

Notes: 1. Li – absolute length of intestine, cm; 2. Lci – the length passed by contrast weight on intestines for 40 min., cm.

Guided by the studied safety of a potential APhI, we decided to continue research in a search for its potential pharmacological activity. In addition, based on existing studies of pharmacological action (Sawczuk et al. 2019; Sidor et al. 2020) and the known composition of the drone homogenate, it is interesting and relevant was the direction of finding its activity as an effective APhI in MetS.

After 8 weeks of use of fructose solution in animals was a small increase in the percentage of weight compared to the control group (9%, $p < 0.001$). Due to the lack of a single result in the scientific literature on the effect of fructose solutions in the experiment, it was important to determine the basal levels of glucose and insulin in experimental animals (Toop et al. 2016).

Fasting blood glucose and insulin levels in control animals at 8 weeks of follow-up were unchanged, and in control animals given 20% fructose solution, basal glucose and insulin levels increased relative to the control group by 103% ($p < 0.001$) and 115% ($p < 0.001$), respectively. It is clear that the increase in the concentration of glucose and insulin in the blood plasma is associated with the consumption of fructose, absorption and metabolism in the liver, which, unlike glucose, is virtually unregulated. Excess fructose intake leads to increased synthesis of fatty acids, which disrupt the signal transmission of insulin to the cell and contribute to the development of hyperinsulinemia and IR (Kazumi et al. 1997; Mamikutty et al. 2014). It is believed that hyperglycemia is a trigger for the activation of various processes that lead to oxidative stress, endothelial dysfunction, the development of atherosclerotic changes and is a major risk factor for macro- and microvascular complications (Gasmi et al. 2020). In addition, there is a linear relationship between hyperglycemia and vascular inflammation. Recent data suggest a role for systemic nonspecific inflammation as a pathogenetic factor linking obesity, insulin resistance, and other components of the MetS (Gasmi et al. 2020).

Although insulin resistance is one of the main results associated with long-term fructose intake (Mamikutty et al. 2014), there is evidence that short-term use of hydrocarbons can induce a transient insulin-resistant state, which can significantly affect insulin-mediated glucose metabolism, contributing to the development of insulin resistance (Toop et al. 2016), which were observed and in our study.

Table 4. Massometric and some biochemical parameters of animal blood under a fructose diet, M ± m (n = 6).

Indicator	Control group	MetS, 20% fructose	MetS + Metformin	MetS+ Lyophilized DBH	Lyophilized DBH
Weight gain in animals, %	38.33 ± 1.202	47.66 ± 1.229	44.50 ± 0.4282	46.83 ± 0.83	37.66 ± 1.16
		$p_1 \le 0.001$	$p_1 \le 0.001$	$p_1 \le 0.001$	$p_1 \ge 0.05$
			$p_2 \ge 0.05$	$p_2 \ge 0.05$	$p_2 \le 0.001$
				$p_3 \ge 0.05$	$p_3 \le 0.001$
					$p_4 \le 0.001$
Glucose, mmol/l	4.69 ± 0.12	9.55 ± 0.23	4.82 ± 0.19	6.98 ± 0.14	5.13 ± 0.11
		$p_1 \le 0.001$	$p_1 \ge 0.05$	$p_1 \le 0.001$	$p_1 \ge 0.05$
			$p_2 \le 0.001$	$p_2 \le 0.001$	$p_2 \le 0.001$
				$p_3 \le 0.001$	$p_3 \ge 0.05$
					$p_4 \le 0.001$
Insulin, μU/ml	8.57 ± 0.14	18.40 ± 023	9.05 ± 0.22	13.93 ± 0.30	9.05 ± 0.29
		$p_1 \le 0.001$	$p_1 \ge 0.05$	$p_1 \le 0.001$	$p_1 \ge 0.05$
			$p_2 \le 0.001$	$p_2 \le 0.001$	$p_2 \le 0.001$
				$p_3 \le 0.001$	$p_3 \ge 0.05$
					$p_4 \le 0.001$
HOMA-IR	1.78 ± 0.07	7.82 ± 0.28	1.94 ± 0.11	4.32 ± 0.11	2.07 ± 0.09
		$p_1 \le 0.001$	$p_1 \ge 0.05$	$p_1 \le 0.001$	$p_1 \ge 0.05$
			$p_2 \le 0.001$	$p_2 \le 0.001$	$p_2 \le 0.001$
				$p_3 \le 0.001$	$p_3 \ge 0.05$
					$p_4 \le 0.001$

Notes, reliability in relation to: p_1 – control; p_2 – MetS, 20% fructose; p_3 – MetS + Metformin; p_4 – MetS+ Lyophilized DBH.

In particular, in animals of control pathology, there was an increase in HOMA-IR by 340% ($p < 0.001$) compared with control group of animals. These changes confirm the proper induction of MetS in our study, which is consistent with previous reports (Mamikutty et al. 2014).

Administration of metformin and lyophilized DBH powder to animals decrease the ($p < 0.001$ in each case): fasting blood glucose by 98% and 37%, respectively, serum insulin by 103% and 32%, respectively, and HOMA-IR index 303% and 81%, respectively, compared with animals that consumed only fructose.

To understand the mechanism of action of lyophilized DBH powder on glucose levels, it is necessary to further study the effect of this APhI on hydrocarbon metabolism. From the analyzed scientific literature, it can be assumed that the glycemia of the lyophilized powder of drone homogenate can be influenced by the flavonoids present in it by inhibiting the absorption of glucose in the intestine or by influencing its absorption by peripheral tissues (Pavliuk et al. 2020; Stechyshyn et al. 2020; Budniak et al. 2021). Flavonoids are known to accelerate the use of glucose in the liver and skeletal muscle cells by activating key glycolysis enzymes hexokinase and pyruvate kinase, reduce glycogen phosphorylase activity and stimulate glycogen production in the liver and skeletal muscle cells (Pavliuk et al. 2020; Shanaida et al. 2020; Stechyshyn et al. 2020).

The presence in the DBH of thiamine, which is an important cofactor of enzymes of glucose and fat metabolism, vitamin E, C, H, carotenoids, copper, potassium, and zinc through various mechanisms can also affect glucose metabolism. It has been proven that the consumption of vitamin E and carotenoids is associated with a reduced risk of developing type 2 diabetes, and the combined use of ascorbic acid with α-tocopherol, β-carotene, N-acetylcysteine, and

selenium in diabetic rats led to lower glucose, HbA1C, and increase in plasma insulin levels, accompanied by a decrease in β-cell apoptosis (Kumar et al. 2013; Sidor et al. 2020).

Conclusion

Thus, as a result of research, we saw that drone brood homogenate from the point of view of pharmacology safety does not have a local irritant effect and does not cause ulceration, does not affect the gastric secretory function and motor-evacuatory activity of the GIT, and is low toxicity confirms the possibility of its long-term safe use. The effect of lyophilized DBH powder on the level of glucose and insulin, which are reflected through one of the main diagnostic markers of MetS – the HOMA index, seems promising in terms of finding new alternative drugs for the prevention and control of MetS.

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