Annals of Pharmacy Practice and Pharmacotherapy
ISSN: 3062-4436
2022, Volume 2, Page No: 98-105

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Preclinical Assessment of the Safety of Drone Brood Homogenate and Validation of Its Pharmacological Effects

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Received: 29 March 2021; **Revised:** 18 August 2022; **Accepted:** 21 August 2022

ABSTRACT

Despite extensive research, the study of metabolic syndrome and its integration with other pathological conditions remains highly relevant. The complex interplay of various pathogenic mechanisms necessitates the development of new diagnostic and therapeutic tools. Drone brood homogenate, a compound with a multifaceted chemical composition, shows considerable promise for contemporary research. Its broad safety profile and applicability across different age groups further highlight its potential as a research subject. This study focused on evaluating the pharmacological safety of drone brood homogenate, including its acute toxicity, effects on gastrointestinal functional and motor activity, local irritation of the gastric mucosa, and influence on gastric secretory function. All experiments were conducted using established classical methods. The specific pharmacological activity of drone brood homogenate was assessed in comparison to metformin in an experimental model of fructose-induced metabolic syndrome. Laboratory animals from the Vivarium of I. Horbachevsky Ternopil National Medical University was used, with all procedures meeting bioethical standards. Results demonstrated that lyophilized drone brood homogenate does not cause local irritation, gastric ulcers, or alterations in gastric secretory function or gastrointestinal motility, indicating its low toxicity and potential for safe long-term use. As expected, animals with induced metabolic syndrome exhibited significant increases in glucose, insulin, and HOMA index. Administration of drone brood homogenate produced a relatively favorable effect on these metabolic parameters. Therefore, drone brood homogenate represents a promising active pharmaceutical agent for correcting biochemical disturbances associated with metabolic syndrome.

Keywords: Drone brood homogenate, Metabolic syndrome, Fructose, Insulin, Glucose

How to Cite This Article: Moreau I, Petit J, Fournier C. Preclinical Assessment of the Safety of Drone Brood Homogenate and Validation of Its Pharmacological Effects. Ann Pharm Pract Pharmacother. 2022;2:98-105. https://doi.org/10.51847/2obOdxFzS0

Introduction

Metabolic syndrome (MetS) remains a critical and socially significant challenge in contemporary medicine, drawing the attention of a diverse range of specialists worldwide, including cardiologists, endocrinologists, general practitioners, geneticists, and therapists [1, 2]. MetS represents a complex cluster of interconnected pathological conditions, encompassing disorders such as diabetes mellitus and cardiovascular diseases [2-4].
As illustrated in **Figure 1**, MetS consists of multiple metabolic abnormalities that reinforce one another, creating a pathologically linked cycle that is challenging to disrupt [3, 5].

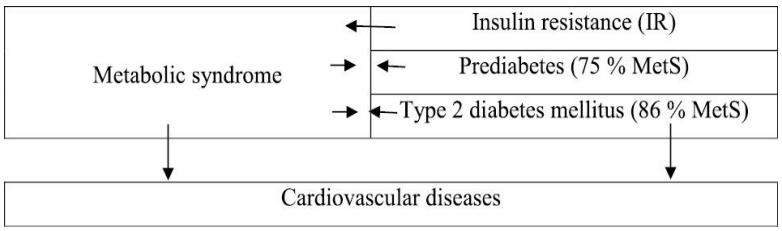

Figure 1. Relationships between Metabolic Syndrome, Insulin Resistance, Pre-diabetes, and Type 2 Diabetes Mellitus

It is widely accepted that the development of metabolic syndrome (MetS) is closely linked to insulin resistance (IR), which initiates a cascade of metabolic disturbances, contributes to severe cardiovascular complications, and has been recognized as an independent risk factor for cardiovascular disease [6-8]. According to current research, IR is characterized by reduced insulin sensitivity, often accompanied by hyperinsulinemia and atherogenic dyslipidemia. Studies indicate a strong correlation between the key components of IR syndrome: the greater the impairment in insulin sensitivity, the higher the levels of insulin, and the greater the risk of associated metabolic disorders. Conversely, the more pronounced the metabolic and functional disturbances, the higher the likelihood of developing IR [6-8].

Given these challenges, there is a clear need to identify novel therapeutic agents that can mitigate the harmful effects of MetS, support tissue regeneration, and demonstrate minimal adverse effects. In this context, natural bee products, particularly drone brood homogenate (DBH), have emerged as promising candidates. DBH has been shown to enhance metabolism during periods of physical activity, improve physical performance, stimulate immune function (including antibody production in the spleen and T-lymphocyte response), and reduce oxidative stress and cardiovascular mortality risk [9, 10]. Its high biological activity stems from a unique combination of bioactive compounds, providing hormone-like effects, rejuvenation, blood pressure regulation, cholesterol reduction, and modulation of metabolic processes [9, 10]. The absence of major contraindications and applicability across a wide age range further underscores its potential as a research subject [10].

Chemically, DBH contains a rich array of proteins, amino acids, nucleic acids, enzymes, phospholipids, mono-, di-, and hydroxycarboxylic acids, fatty acids, steroid hormones, carbohydrates, flavonoids, essential micro- and macronutrients, and water- and fat-soluble vitamins (A, D, E, PP, C, and B complex), among many other biologically active substances [10-12].

The diverse pharmacological profile of DBH allows it to target multiple aspects of MetS. For example, phytosterols present in DBH exert endocrine-stimulating effects without inducing hormonal imbalance and play a critical role in managing dyslipoproteinemia, a key factor in MetS progression. Structurally similar to cholesterol, phytosterols bind to low-density lipoproteins, preventing the formation of more atherogenic compounds. Regular intake of phytosterols can reduce LDL and total cholesterol levels by 10–15%, with long-term studies showing sustainable cholesterol-lowering effects over periods up to 85 weeks [13-17].

Phytosterols in DBH also contribute to anabolic activity, supported by amino acids, trace elements, and vitamins A and B. The B vitamins further enhance protein, fat, and carbohydrate metabolism and support the synthesis of acetylcholine, a key neurohumoral mediator that regulates nitric oxide (NO) production [18, 19]. Disruption of NO synthesis is a hallmark of endothelial dysfunction, an independent factor in the onset and progression of microvascular pathology.

Among DBH components, 10-oxo-2-decenoic acid is particularly important, as it binds excess peroxides associated with MetS, directly mitigating oxidative stress [1, 5, 11]. Oxidative stress, resulting from endothelial dysfunction and depleted antioxidant defenses, is considered a central mechanism in the development of MetS complications [3]. Hyperglycemia-induced oxidative stress also promotes β-cell damage. Flavonoids in DBH provide anti-inflammatory effects that further enhance its antioxidant properties [12].

Additionally, DBH boosts metabolic rate during physical activity, thereby improving endurance and overall physical performance [20, 21].

Materials and Methods

Before evaluating specific pharmacological effects, it is essential, as per the State Export Center (SEC) of the Ministry of Health of Ukraine (MHU) guidelines on “Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals,” to examine the safety pharmacology of the active pharmaceutical ingredient (APhI), in this study, lyophilized DBH powder. This assessment helps identify any unexpected pharmacological effects and allows detailed monitoring during toxicology studies if necessary. Safety pharmacology focuses on functional parameters that reveal potential toxic effects, aiding in the identification of organ-specific toxicity mechanisms relevant to human use or clinical indications [22, 23].

To determine the relationship between administered dose and systemic or local toxicity, a single-dose toxicity study was carried out. Acute toxicity of lyophilized DBH powder was tested according to SEC recommendations [22] on adult Wistar rats of both sexes (180–220 g body weight). The test compound (LLC “Natural Beauty,” Ukraine) was delivered as a single aqueous dose corresponding to toxicity class IV (“Slightly toxic”) at 5000

mg/kg. Rats were maintained on a standard diet with free access to water, and general health, behavior, and physical condition were observed daily over a 14-day period. Toxicity levels were classified using the Hodge and Sterner scale [24].

For substances intended for repeated administration, local irritation must also be evaluated. Therefore, the effects of lyophilized DBH on gastrointestinal motility, functional activity, and the gastric mucosa were investigated. The potential ulcerogenic effect was studied in Wistar rats of both sexes (200–220 g) following a 24-hour fast with unrestricted water access [25, 26]. Lyophilized DBH (72 mg/kg) was administered intragastrically, and acetylsalicylic acid (ASA, 100 mg/kg, Bayer Bitterfeld GmbH) served as the reference. Four hours later, under sodium thiopental anesthesia, the rats were euthanized, and the stomach mucosa was examined with magnification. Lesions were scored on a scale of 0–5: 0, no damage; 1, 1–3 small ulcers; 2, more than three small ulcers; 3, a major ulcer plus several minor ulcers; 4, multiple large ulcers; 5, perforated ulcer with bleeding. Early mucosal changes (edema, redness, vascular injection, hemorrhage) were scored as 0.5 points. The proportion of animals exhibiting gastric or intestinal lesions was recorded.

Ulcer index (UI) was calculated by the formula [27]:

UI = (degree of ulcer * percentage of animals with ulcers)/100.

To evaluate the influence of lyophilized DBH on gastrointestinal function, adult rats weighing 180–220 g were examined for gastric secretory activity following the methodology of Andreeva A.I. and Sharova S.A. [28]. Prior to the experiment, animals were fasted for 24 hours. One hour after intragastric administration of lyophilized DBH at a conditional therapeutic dose of 72 mg/kg, both experimental and control rats were anesthetized. The pyloric sphincter was then ligated, and after 4 hours, the cardiac end of the stomach was also ligated. Gastric juice was collected and its volume measured, with secretion intensity calculated per 100 g of body weight. Total and free gastric acidity were determined via titration with 0.1 M NaOH using phenolphthalein and bromothymol blue indicators, expressed as the volume of NaOH required to neutralize 100 mL of gastric juice. Bound acidity was calculated as the difference between total and free acidity.

The effect of lyophilized DBH on gastrointestinal motility was assessed in white mice weighing 21–23 g, following Sticknay J.S. *et al*. [29]. Animals were fasted for 24 hours with unrestricted water access. Mice in the experimental group received lyophilized DBH at a conditional therapeutic dose, while controls were given an equal volume of water. After 1 hour, all mice were administered 0.3 mL of a contrast medium (10% activated carbon in 1% starch paste) intragastrically. Forty minutes later, under thiopental anesthesia, the animals were sacrificed, and both the total intestinal length and the distance traveled by the contrast medium were measured. Peristaltic activity was expressed as the percentage of the intestinal length traversed by the contrast relative to the full intestinal length.

The scope of pharmacological evaluation of lyophilized DBH powder was determined according to the SEC of the MHU guidelines and the available scientific data [22]. Based on existing knowledge of the active pharmaceutical ingredient, its effects were further examined in a metabolic syndrome (MetS) model. Adult male Wistar rats (230–260 g) were randomly allocated into five groups (n = 6): Group 1 – healthy control, Group 2 – control pathology (CP), Group 3 – treated with metformin (SANDOZ, 500 mg, LEK, Poland) at 60 mg/kg, Group 4 – treated with lyophilized DBH powder (LLC “Natural Beauty,” Ukraine) at 72 mg/kg, and Group 5 – also treated with lyophilized DBH powder at 72 mg/kg. Groups 2, 3, and 4 received a 20% fructose solution instead of water for 8 weeks to induce MetS [30, 31].

This 2-month fructose regimen simulates insulin resistance in target tissues, approximating six human years of daily fructose intake [32]. Unlike high-fructose models (60–70%), this concentration better represents the human insulin resistance profile while still inducing major metabolic disturbances in rats [33]. From week 6 of MetS modeling, Group 3 received metformin and Group 4 received lyophilized DBH intragastrically for 14 days in a combined therapeutic and prophylactic protocol.

All animals were maintained under standard vivarium conditions at I. Horbachevsky Ternopil National Medical University, housed individually under a 12-hour light/dark cycle, with unrestricted access to food and water. All procedures adhered to national and international guidelines for the care and use of laboratory animals [34, 35].

Fasting blood glucose was measured using the FreeStyle Optium device. Hepatic and peripheral tissue sensitivity to insulin was assessed via a short insulin test. Insulin resistance was evaluated using the Homeostasis Model Assessment (HOMA), which mathematically quantifies insulin-glucose interactions.

Calculation of insulin resistance

Insulin resistance was evaluated by calculating the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index using the formula:

HOMA-IR index = [fasting glucose (mmol/L) × fasting insulin (μU/ml)]/22.5 as described by Matthews *et al*. (1985) [36].

The data obtained were analyzed using methods of variation statistics. For each parameter, the arithmetic mean and standard error were calculated. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied to assess statistical significance. All analyses were conducted using GraphPad Prism, version 5.0 (GraphPad Software, Inc.).

Results and Discussion

Preclinical evaluation of the safety pharmacology of lyophilized DBH powder was carried out following Good Laboratory Practice principles. The test substance was administered once intragastrically as an aqueous solution at the maximum dose corresponding to toxicity class IV ("Slightly toxic"), i.e., 5000 mg/kg. Throughout the observation period, no signs of intoxication or physiological disturbances were noted in the rats: animals remained active, maintained normal appetite, urination, and defecation, and exhibited a clean and healthy appearance comparable to the control group. Reflex responses were preserved in all animals, and the total number of animals remained unchanged at six per group.

Upon termination of the experiment and macroscopic examination, critical organs and systems showed no observable alterations. Accordingly, following the SEC of the MHU guidelines and the Hodge and Sterner toxicity scale (1943), lyophilized DBH powder can be classified as toxicity class IV, "Slightly toxic."

Examination of the gastric mucosa and duodenum revealed that lyophilized DBH, at all tested doses, did not induce damage to the gastrointestinal lining, in contrast to the pronounced lesions observed in animals treated with acetylsalicylic acid **(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	4.68 ± 0.12
	p ≤ 0.001	p ≤ 0.001

In contrast to rats receiving acetylsalicylic acid at 100 mg/kg, which showed gastric lesions, administration of lyophilized DBH produced no ulcer formation, supporting its potential safety for oral application **(Table 1)**.

Assessment of the drone homogenate's impact on gastric secretory activity revealed that a conditional therapeutic dose of 72 mg/kg did not alter gastric juice volume or modify free, total, or bound acidity **(Table 2)**. These findings indicate that lyophilized DBH does not exert any measurable effect on the stomach's secretory function.

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	89.35 ± 0.98	11.06 ± 0.64
		p ≥ 0.05	p ≥ 0.05	p ≥ 0.05

An important aspect of evaluating the pharmacological profile of orally administered drugs is their potential impact on gastrointestinal motility. Experimental results indicated that administration of lyophilized DBH at 72

mg/kg had no effect on the transit of the activated charcoal suspension through the intestine **(Table 3)**. Consequently, intestinal peristaltic activity in animals treated with the drone brood homogenate remained comparable to that of the control group.

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.

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
		$p_1 \leq 0.001$	$p_1 \leq 0.001$	$p_1 \leq 0.001$	$p_1 \geq 0.05$
			$p_2 \geq 0.05$	$p_2 \geq 0.05$	$p_2 \leq 0.001$
				$p_3 \geq 0.05$	$p_3 \leq 0.001$
					$p_4 \leq 0.001$
Glucose, mmol/l	4.69 ± 0.12	9.55 ± 0.23	4.82 ± 0.19	6.98 ± 0.14	5.13 ± 0.11
		$p_1 \leq 0.001$	$p_1 \geq 0.05$	$p_1 \leq 0.001$	$p_1 \geq 0.05$
			$p_2 \leq 0.001$	$p_2 \leq 0.001$	$p_2 \leq 0.001$
				$p_3 \leq 0.001$	$p_3 \geq 0.05$
					$p_4 \leq 0.001$
Insulin, μU/ml	8.57 ± 0.14	18.40 ± 023	9.05 ± 0.22	13.93 ± 0.30	9.05 ± 0.29
		$p_1 \leq 0.001$	$p_1 \geq 0.05$	$p_1 \leq 0.001$	$p_1 \geq 0.05$
			$p_2 \leq 0.001$	$p_2 \leq 0.001$	$p_2 \leq 0.001$
				$p_3 \leq 0.001$	$p_3 \geq 0.05$
					$p_4 \leq 0.001$
HOMA-IR	1.78 ± 0.07	7.82 ± 0.28	1.94 ± 0.11	4.32 ± 0.11	2.07 ± 0.09
		$p_1 \leq 0.001$	$p_1 \geq 0.05$	$p_1 \leq 0.001$	$p_1 \geq 0.05$
			$p_2 \leq 0.001$	$p_2 \leq 0.001$	$p_2 \leq 0.001$
				$p_3 \leq 0.001$	$p_3 \geq 0.05$
					$p_4 \leq 0.001$

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

Guided by the favorable safety profile of the tested APhI, further investigations were undertaken to explore its potential pharmacological effects. Given previous research on its bioactivity [9, 10] and the known composition of drone brood homogenate, examining its efficacy in the context of metabolic syndrome (MetS) appeared both relevant and timely.

Following 8 weeks of fructose administration, animals exhibited a modest weight gain relative to controls (9%, $p < 0.001$). Due to limited consistency in the literature regarding experimental fructose effects, basal glucose and insulin levels were assessed to establish baseline metabolic parameters [37]. In control animals, fasting glucose and insulin remained stable over the 8-week period, whereas rats receiving 20% fructose demonstrated significant increases in basal glucose and insulin levels, rising by 103% ($p < 0.001$) and 115% ($p < 0.001$), respectively. These elevations are attributable to fructose metabolism in the liver, which bypasses regulatory mechanisms affecting glucose, promoting fatty acid synthesis, impairing insulin signaling, and contributing to hyperinsulinemia and insulin resistance [38, 39]. Hyperglycemia can trigger oxidative stress, endothelial dysfunction, atherosclerotic changes, and is a key risk factor for both macrovascular and microvascular complications [40]. Moreover, hyperglycemia correlates with vascular inflammation, highlighting the role of systemic nonspecific inflammation in linking obesity, insulin resistance, and other components of MetS [40].

While chronic fructose intake is widely associated with insulin resistance [39], short-term exposure can also induce a transient insulin-resistant state, impairing insulin-mediated glucose metabolism [37], which was observed in our study. Specifically, animals in the control pathology group exhibited a 340% increase in HOMA-IR ($p < 0.001$) relative to healthy controls, confirming effective MetS induction, consistent with prior reports [39].

Administration of metformin and lyophilized DBH powder significantly reduced metabolic markers: fasting blood glucose decreased by 98% and 37%, serum insulin by 103% and 32%, and HOMA-IR by 303% and 81%, respectively, compared to animals receiving fructose alone.

To elucidate the mechanism underlying lyophilized DBH's glucose-lowering effects, further investigation into carbohydrate metabolism is warranted. Evidence suggests that flavonoids present in the homogenate may reduce glucose absorption in the intestine or enhance peripheral tissue uptake [1, 3, 41]. Flavonoids are also known to promote glucose utilization in the liver and skeletal muscle by activating key glycolytic enzymes, including hexokinase and pyruvate kinase, suppress glycogen phosphorylase activity, and stimulate glycogen synthesis in hepatic and muscle tissues [1, 3].

Additional components of DBH, such as thiamine (a cofactor in carbohydrate and lipid metabolism), vitamins E, C, H, carotenoids, and trace elements including copper, potassium, and zinc, may further modulate glucose homeostasis. Intake of vitamin E and carotenoids has been associated with a lower risk of type 2 diabetes, and combined supplementation of ascorbic acid with α-tocopherol, β-carotene, N-acetylcysteine, and selenium in diabetic rats reduced blood glucose and HbA1C while increasing plasma insulin and reducing β-cell apoptosis [10, 42].

Conclusion

The study demonstrates that drone brood homogenate exhibits a favorable safety profile: it lacks local irritant or ulcerogenic effects, does not interfere with gastric secretory function or gastrointestinal motility, and shows low toxicity, supporting its potential for safe long-term use. Furthermore, lyophilized DBH powder effectively modulates glucose and insulin levels, as reflected in improvements in HOMA-IR, highlighting its promise as a potential alternative therapeutic agent for the prevention and management of MetS.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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