STATE OF HYPERGLYCEMIA ANIMALS IN THE CONSUMPTION OF HIGH-CALORIE DIET WITH THE BIONANOCOMPOSITE ADDITION

Glucose level and glucose tolerance test in blood of rats under conditions of obesity induced by consumption of high-calorie diet have been determined. It was also researched these indicators in the blood of animals that consumed high-calorie diet with the bionanocomposite addition under the possibility of the state of hyperglycemia.

Key words: glucose, glucose tolerance test, bionanocomposite, prevention, obesity.

Introduction. Obesity is a significant health problem worldwide and its incidence has more than doubled during the last 20 years [1]. Obesity is characterized by both a large increase in body weight and an increase in body fat exceeding standard measures. Obesity is a leading risk factor for the development of cardiovascular disease, and malignancies, and also has an impact on respiratory diseases such as asthma, chronic obstructive pulmonary disease as well as obesity hypventilation syndrome and sleep apnea [2-4]. Obesity is associated with most of the components of metabolic syndrome, the leading cause of type 2 diabetes. The comorbidities of obesity and type 2 diabetes associated with hyperglycemia (high blood glucose and impaired glucose tolerance). Therefore determination of glucose and glucose tolerance test is an important in period of prevention or treatment obesity.

Features of metabolism at obesity can be the basis for the study of biologically active compounds of plant origin for the prevention of pathology. There is greater need to study the pharmacological and toxicological effects of herbal products to examine their clinical efficacy and safety. Because, every drug has potential side effects. They are not completely safe in this regard. Herbal supplements are being extensively used due to their effectiveness in managing many chronic disorders. They are cost-effective, and exert less to no toxic side-effects in comparison with many chemically synthesized drugs [5].

Out of many such medicinal plants, fenugreek (Trigonella foenum-graecum Linn (Fabaceae)) has recently attracted the attention of scientists from across the globe. Fenugreek belongs to the family Fabaceae and is applied in many parts of the world for the treatment of diabetes. Fenugreek is well known for its anti diabetic properties [6-9]. The plant has been employed against different diseases including diabetes.

In previous studies we have shown development of prediabetes in rats maintained on a high-calorie diet [10-12]. The aim of this work was to compare the performance of prediabetes in male and female under development of obesity and the impact of prophylactic administration bionanocomposite under the possibility of the state of hyperglycemia.

Materials and methods.

Animal care and experimental procedures

Experiments were carried out on outbred female and male rats initially weighing 150–170 g. Research was conducted according to with the standards of the Convention on Bioethics of the Council of Europe’s Europe

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Convention for the Protection of Vertebrate Animals' used for experimental and other scientific purposes' (1997), the general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001) and other international agreements and national legislation in this field. Animals were kept in a vivarium that was accredited in accordance with the 'standard rules on ordering, equipment and maintenance of experimental biological clinics (vivarium)'. The tools used to research were metrological control.

During week 1, all animals received standard Purina rodent chow and water ad libitum. On day 8, the animals were randomized into 4 groups. Rats of group 1 (Control) were given water ad libitum and were fed by a standard food during 14 weeks of the experimental period. The (HCD) group was fed by a high-caloric diet and water ad libitum [13]. Rats of group 3 (Control_BNC) were fed by a standard nutriment supplemented with 2 % bionanocomposite based on fenugreek seed powder. The (HCD_BNC) group was fed by a high-caloric diet which contained bionanocomposite based on fenugreek seed powder (2 %) during 14 weeks of the experimental period.

The animals were housed individually in cages at a constant temperature (20-22 °C) and humidity (60 %) with a 12-hour-light and 12-hour-dark cycle.

**Intraperitoneal glucose tolerance testing**

The serum glucose concentration was monitored using intraperitoneal glucose tolerance testing (IPGTT) in the control and obese rats. Before the test, the animals were narcotized with sodium thiopental (40 mg/kg body weight intraperitoneally). The rats were injected intraperitoneally with glucose solution (2 g/kg body weight). Blood samples were drawn from the tail vein at 0, 15, 30, 60, and 120 minutes after glucose administration through an intravenous catheter. Blood glucose levels were measured using a glucometer (Accu-Chek-Performa, Roche Diagnostics, Penzberg, Germany). The obtained data were used for construction of glycemic curves that reflect the rate of glucose level normalization after glucose administered.

**Statistical analysis**

Statistical analysis of data was carried out by the software package 'Statistica 7.0'. For the analysis of data distribution type, Shapiro-Wilks criterion was used. As the data were normally distributed, we used Student's t test for independent samples. Mean values (M) and standard deviations (SD) were calculated. Significant difference was considered at $p \leq 0.05$.

**Results and discussion.**

Prediabetes is defined as an intermediate metabolic state between normoglycaemia and diabetes. Prediabetes includes impaired glucose tolerance and impaired fasting glucose [14, 15]. The blood glucose concentration in control and experimental groups of rats are represented in Fig 1.

**Fig. 1. Effect of BNC on blood glucose in normal and obese rats (A-female, B-male)**

Means ± standard errors ($n = 6$) are shown (* $p < 0.05$ in comparison with control group; ** $p < 0.05$ in comparison with HCD group).

Blood glucose concentration in control female and male rats was significantly higher than in high-calorie diet group (Fig 1, A,B). Blood glucose concentration in control female rats was 3.4 mmol/L (in control male rats was 3.6 mmol/L), while feeding high-calorie diet led to 1.7-fold elevation (5.8 mmol/L) (in male – to 1.6-fold elevation (5.8 mmol/L)) of fasting blood glucose (Fig. 1,A,B). In the group of obese male rats treated with BNC had significantly lower blood glucose level compared to untreated obese rats. Blood glucose concentration in male rats of HCD_BNC group was 3.3 mmol/L (Fig. 1, B). The results suggest a positive impact BNC on key indicator of diabetes and prediabetes. The blood glucose levels in control male and female rats treated with BNC and in untreated control animals were statistically comparable (Fig. 1, A,B).

Our results indicate the development of hyperglycemia in animals research group. Hyperglycemia can develop due to decreased insulin secretion by β-cells of the pancreas, as well as lack of glucose utilization body tissues. High levels of glucose in the blood and other biological fluids causes the development of osmotic diuresis, which leads to dehydration and deficiency of cations. In addition, elevated levels of glucose increases non-enzymatic glycosylation of proteins and lipids and consequently develop numerous lesions in various organs [256]. However, adding a bionanocomposite in HCD prevents the development of hyperglycemia.
Effect of BNC on glucose tolerance in normal and obese rats is shown in Fig. 2.

Glucose administration induced a high glucose level in the obese animals at all time points, from 0 to 120 minutes after glucose challenge, and the difference was significant at the 15 minutes and 30 minutes time point.

For the control female rats, the mean blood glucose level was 4.03 mmol/L at 30 minutes after glucose administration and 3.45 mmol/L at 120 minutes (Fig. 2A). For the control male rats, the mean blood glucose level was 4.7 mmol/L at 15 minutes after glucose administration and 3.4 mmol/L at 120 minutes (Fig. 2B). Although impaired glucose tolerance is consistently defined as a 2 hour plasma glucose concentration of 7.8-11.0 mmol/L during an oral glucose tolerance test, the cut-off point for diagnosis of impaired fasting glucose remains controversial [14]. WHO defines impaired plasma glucose as fasting plasma glucose of 6.1-6.9 mmol/L [14, 15], while the 2003 American Diabetes Association (ADA) guideline recommended a cut-off point of 5.6-6.9 mmol/L [14, 16]. For the obese female (and male) rats, the mean blood glucose level was 6.4 mmol/L (7.1 mmol/L) at 15 minutes after glucose administration and 6.0 mmol/L (6.2 mmol/L) at 120 minutes (Fig. 2A). IP glucose tolerance tests showed that HCD fed rats had impaired glucose clearance compared to standard food fed rats, as indicated by a significantly greater area under the glucose curve (Fig. 2A).

Prediabetes defined as impaired fasting glucose or impaired glucose tolerance is associated with an increased risk of composite cardiovascular events, coronary heart disease, stroke, and all cause mortality. In this regard, during the prevention of obesity should pay attention not only to normalize body weight but in maintaining glucose at normal levels. In groups of animals (Control_BNC and HCD_BNC) were observed glucose intolerance. Such data provide basis for further study of the bionanocomposite as a possible means for prevention and treatment of obesity.

Fig. 2. Effect of BNC on glucose tolerance in normal and obese rats (A-female, B-male)

Top panels show plasma glucose concentration curves as a function of time, and bottom panels show areas under the curves. Means ± standard errors (n = 6) are shown ( * p < 0.05 in comparison with control group; ** p < 0.05 in comparison with HCD group).

References
СТАН ГІПЕРГЛІКЕМІЇ ТВАРИН

В УМОВАХ СПОЖИВАННЯ ВИСОКОКАЛОРЕЙНОЇ ДІЄТИ

З ДОДАВАННЯМ БІОНАНОКОМПОЗИТУ

Визначено вміст глюкози в крові щільності і проведено глюкозотолерантний тест за умов зміни обійки, індукуваного спожи- 
ванням висококалорійної дієти. Досліджено дані показники у крові тварин, які споживали висококалорійну дієту з додаванням біонано- 
композиту. Отримані дані свідчать про профілактичний ефект біонанокомпозиту на розвиток основних показників передіабету.

Ключові слова: глюкоза, глюкозо-тOLERANTний тест, біонанокомпозит, профілактика, ожиріння.

СОСТОЯНИЕ ГИПЕРГЛИКЕМИИ ЖИВОТНЫХ

В УСЛОВИЯХ ПОТРЕБЛЕНИЯ ВЫСОКОКАЛОРИЙНОЙ ДИЕТЫ С ДОБАВЛЕНИЕМ БИОНАНОКОМПОЗИТА

Было определено содержание глюкозы в крови крыс и проведен глюкозотолерантный тест в условиях развития ожирения, инду- 
цированного потреблением высококалорийной диеты. Также были исследованы данные показатели в крови животных, которые по- 
требляли высококалорийную диету с добавлением бионанокомпозита. Полученные данные свидетельствуют о профилактическом 
эффекте бионанокомпозита на развитие основных показателей передиабета.

Ключевые слова: глюкоза, глюкозо-толерантный тест, бионанокомпозит, профилактика, ожирение.