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ASSESSMENT OF GLUCOSE AND GLYCOGEN CONTENT AFTER ADMINISTRATION OF AQUEOUS EXTRACT FROM LEMNA MINOR FROND IN EXPERIMENTAL HYPOTHYROIDISM

Experimental study of the evaluation of the effectiveness of the aqueous extract from Lemna minor frond on carbohydrate metabolism in hypothyroid rats was carried out. Experimental hypothyroidism in rats was induced by introduction of a 1 % solution of sodium perchlorate instead of drinking water for 20 days. After 20 days, the experimental group of animals with hypothyroidism was treated with aqueous extract from Lemna minor frond at a dose of 0.5 ml/100 g body weight and with reference drug lodomarin at a dose of 12 mg iodine/kg. The results of studies has shown evident corrective effect of aqueous extract from Lemna minor frond on synthetic thyroid function and carbohydrate metabolism in hypothyroidism, which manifested itself in the normalization of thyroid hormones, increasing glucose in blood serum and reducing liver glycogen content. Thus, the aqueous extract from Lemna minor frond can be attributed to hypothyroid state and carbohydrate metabolism regulators and is promising for its effectiveness further study.

Key words: aqueous extract from *Lemna minor* frond; experimental hypothyroidism; sodium perchlorate; thyroid hormones; glucose; glycogen

INTRODUCTION

Metabolic processes controlled by the neuroendocrine system. Such control supports body homeostasis and provides fast response to changes from an external environment, contributing to effective adaptation. Violation of the functional activity of any endocrine glands leads to changes in metabolism and, consequently, an imbalance in the homeostasis system. Hormones were synthesized by thyroid gland - thyroxine (T₄) and triiodothyronine (T₂) which affect a wide range of metabolic and physiological processes play an important role in the adaptation. Thyroxin and a lesser extent triiodothyrinine regulate a variety of biochemical reactions in virtually all tissues. These hormones are known as important factors in gene regulation in tissues such as brain, liver, muscles and adipose tissue. They are involved in the control of resting metabolism. Thyroid hormone status is also important for glucose homeostasis. Hypothyroidism reduces the capacity for glucogenesis, in part, by lowering hepatic glycogen synthesis [1, 2, 4].

Hormone therapy used in hypothyroidism, corrects various symptoms, including violations of metabolic processes, through artificial restoration of thyroid hormones blood levels. One of the promising ways to improve the treatment of hypothyroidism is used of herbal medicines,

which have pharmacodynamic properties enhance the effect of basic medicines, aimed at addressing the major manifestations of the disease and improved the safety level of their destination long courses [3, 5, 10]. One of the herbs that contains a set of biologically active substances and can be effective in hypothyroidism is *Lemna minor*. Phytochemical studies have shown that *Lemna minor* frond contains phytosterols, saturated hydrocarbons, aldehydes and ketones, fatty acids, amino acids, iodine compounds, macro- and microelements and other biologically active substances [7, 12].

Taking this to account, the aim of this work was studied the effect of aqueous extract from *Lemna minor* found in carbohydrate metabolism parameters in rats with experimental hypothyroidism.

MATERIALS AND METHODS

Experiments were conducted on nonlinear white rats weighing 120-150 g. The study protocol was approved by Bioethics Commission of the National University of Pharmacy and the "General ethical animal experimentation" (Kyiv, 2001), consistent with the provisions of the European Convention for the Protection of Vertebrate Animals were used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Experimental hypothyroidism was induced by sodium per chlorate in distilled drinking water (1 g per liter)

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Table

THE CONCENTRATION OF THYROID HORMONES (T_3 , T_4) IN SERUM RAT RESEARCH GROUPS (n = 10)

Index	Control	Hypothyroid	Treated with AELMF	Treated with Iodomarin
T ₃ , nmol/l	2.59 ± 0.20	1.36 ± 0.07*	2.13 ± 0.16**	1.97 ± 0.104*/**
T ₄ , nmol/l	63.37 ± 3.03	27.49 ± 1.32*	51.50 ± 2.61*/**	45.85 ± 1.62*/**

Note: AELMF – aqueous extract from $Lemna\ minor$ frond; Values are mean \pm SEM, n = number of animals in each group; * – P < 0.05 versus control group; ** – P < 0.05 versus hypothyroid group.

for 20 days. Control (vehicle) rats were received equal volume of drinking water [11]. Twenty days after induction of hypothyroidism treatment of animals was starting. Healthy rats were used as normal control. Rats were induced by hypothyroidism and randomly divided into four groups (n = 10). Group 1 is normal untreated rats were given distilled water 0.5 ml/100 g of body weight daily orally for 21 days. Group 2 is hypothyroid untreated rats were given normal saline 0.5 ml/100 g of body weight daily orally for 21 days. Group 3 is hypothyroid rats were treated with aqueous extract from *Lemna minor* frond 0.5 ml/100 g of body weight daily orally for 21 days. Group 4 is hypothyroid rats were treated with Iodomarin 12 μ g/kg daily orally for 21 days.

After the experiment, animals were sacrificed by immediate decapitation and the blood and the liver were collected. Concentration of T_3 and T_4 determined in blood serum by ELISA was using test kits "Chema" (Russia). Glycogen content was determined in liver homogenate using anthrone reagent [8].

All data expressed as Mean \pm SEM and data were entered and analyzed the using of statistical package "Statistica 6.0" with multiple comparisons. The Newman-Keylsa test was used to determine difference between groups. Values of P < 0.05 were considered as statistically significant [6, 9].

RESULTS AND DISCUSSION

The ELISA results of the blood serum in rats have shown that the use of sodium per chlorate was resulted in decreased synthetic thyroid function, which was shown to reduce the levels of thyroid hormones compared with control group of animals (Table). Thyroxin level was de-

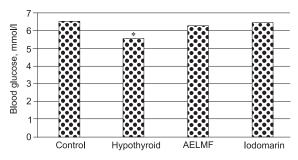


Fig. 1. The content of glucose in the blood serum of rats of different experimental groups

Note: * – P < 0.05 versus control group; AELMF – aqueous extract from Lemna minor frond

creased by 56.6 % (p \leq 0.05), T $_3$ – by 47.5 % (p \leq 0.05). Development hypothyroid condition was also accompanied by changes in glucose homeostasis. The concentration of glucose in the blood serum was decreased by 14.4 %, liver glycogen increased by 2.54 folds (Fig. 1, 2). Decreased glucose level may be connected with changes in its intestine absorption by decreasing hexokinase activity and violation of its transport through the blood. In addition, hypothyroidism associated with decreased gluconeogenesis and glycogenolisis resulting in increment in glycogen in liver. In addition, hypothyroidism lowers glycogen phosphorylase activity in the liver [2, 4].

The introduction of aqueous extract from *Lemna minor* frond led to improvement of synthetic thyroid function, which was resulted in increasing of T_4 and T_3 levels by 1.87 and 1.56 folds, respectively, compared with hypothyroid animals. It should be noted that effect of study extract on thyroid hormones was slightly higher than the effect of the reference drug Iodomarin. Administration of Iodomarin led to the increasing of T_4 and T_3 by 1.7 and 1.45 folds, respectively, compared with a hypothyroid group (Table).

Analysis of the experimental data relative to glucose in the blood serum of the rats showed that its concentration increased in hypothyroid animals treated with aqueous extract from *Lemna minor* frond and reached the level of control group. Effect of the reference drug Iodomarin, which was used for the prevention of thyroid diseases due to lack of iodine, also resulted in the increasing of blood glucose until values of control group.

Experimental data has shown that investigated objects act on liver glycogen content in rats in the same manner as on the thyroid hormones level. Course introduction

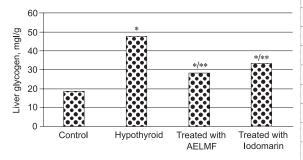


Fig. 2. The content of glycogen in the liver of rats of different experimental groups

Note: * – P < 0.05 versus control group; ** – P < 0.05 versus hypothyroid group; AELMF – aqueous extract from Lemna minor frond

of aqueous extract from Lemna minor frond has resulted in normalizing liver function in rats, which manifested itself in a significant increased glycogen levels by 41.3 % (p < 0.05) compared with a hypothyroid group. In addition, the experiment has shown that the effect of investigated extract on the glycogen content was slightly higher than the effect of the reference drug Iodomarin. The use of which led to the likely increase in glycogen content by 30.9 % was compared with the hypothyroid group. Based on obtained experimental data may suggest that the effect of aqueous extract from Lemna minor frond was not only realized through its action on thyroid function, but also due to its effects on enzyme systems was involved in the glucose metabolism. Aqueous extract from Lemna minor frond mechanism of action on these processes requires further study.

CONCLUSIONS

Experimental hypothyroidism was induced by administration of 1 % sodium perchlorate solution, characterized by reduced synthetic thyroid function and, consequently, changes in carbohydrate metabolism, manifested in the decreasing of $T_{\rm 4}$ and $T_{\rm 3}$ levels, the concentration of blood serum glucose and increasing the liver glycogen content in the experimental animals. The introduction of aqueous extract from *Lemna minor* frond at a dose of 0.5 ml/100 g body weight contributed to raising the thyroid hormones and glucose levels and reduced glycogen content in animals with experimental hypothyroidism. Thus, the aqueous extract from *Lemna minor* frond can be attributed to thyroid hypofunction correctors and is promising for further study of its efficacy and mechanisms of action in other models of experimental hypothyroidism.

REFERENCES

- Boelen A. Thyroid hormones and glucose metabolism: the story begins before birth // Exp. Physiol. 2009. Vol. 94. P. 1050-1051.
- Chakrabarti S. Thyroid disfunction modulates glucoregulatory mechanism in rat / S. Chakrabarti, S. Gu-

- ria, I. Samanta, M. Das // Ind. J. Exp. Biology. 2001. Vol. 45. P. 549-553.
- 3. Fadeev V. V. Problems of substitution treatment of hypothyroidism: present and prospects // Clin. i Experiment. Thyroidol. 2012. Vol. 8 (3). P. 12-17.
- Hvojnitskaya L. G. Poll of biochemical indicators of carbohydrate, lipid and electrolyte metabolism during phytotherapy of experimental hyperthyroidism / L. G. Hvojnitskaya, A. N. Ryabkov // Russian Biomed. Gazette Academician Pavlov. – 2003. – Vol. 1-2. – P. 42-47.
- Javed Z. Levothyroxine treatment of mild subclinical hypothyroidism: a review of potential risks and benefits / Z. Javed, Th. Sathyapalan // Ther. Adv. Endocrinol. Metab. 2016. Vol. 7 (1). P. 12-23. Doi: 10.1177/2042018815616543.
- 6. Khalaphyan A. A. STATISTICA 6: Statistical analysis of data. M.: Binom-Press, 2007. 512 p.
- Nikiphorov L. A. Study of amino acid composition of duckweed (*Lemna minor* L.) / L. A. Nikiphorov, M. V. Belousov, N. S. Fursa // Bull. Sibirskoy Medicini. – 2011. – Vol. 5. – P. 74-77.
- 8. Meshkova N. P., Severun S. E. Practicum in biochemistry. Determination of glycogen with anthrone. Publishing house of the Moscow University, 1979. P. 41-43.
- Rebrova O. Y. Statistical analysis of medical data. The application package STATISTICA applications. – M.: Media Sphere, 2006. – 312 p.
- Tretyak S. I., Hryschanovich V. Y. Modern methods of treatment of hypothyroidism. – Minsk: BGMU, 2011. – 150 p.
- 11. Yu K. The pharmacokinetics of perchlorate and its effect on the hypothalamus-pituitary-thyroid axis in the male rat / [K. Yu, L. Narayanan, D. Mattie et al.] // Toxicol. Appl. Pharmacol. 2002. Vol. 182 (2). P. 148-159.
- Vladymyrova I. N. Biologically active substunces of *Lemna minor* S. F. gray / I. N. Vladymyrova, V. A. Ge- orgiyants // Himiko-Farmatsevticheskiy zhurn. – 2013. – Vol. 47 (11). – P. 29-31.

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ОЦІНКА ВМІСТУ ГЛЮКОЗИ ТА ГЛІКОГЕНУ ПРИ ЗАСТОСУВАННІ ВОДНОГО ЕКСТРАКТУ ЛИСТЕЦЯ РЯСКИ НА ТЛІ ЕКСПЕРИМЕНТАЛЬНОГО ГІПОТИРЕОЗУ

Проведені експериментальні дослідження з оцінки ефективності впливу водного екстракту листеця ряски малої на показники вуглеводного обміну в умовах гіпотиреоїдного стану у щурів. Експериментальний гіпотиреоз у щурів викликали введенням 1 % розчину перхлорату натрію замість питної води протягом 20 днів. Через 20 днів експериментальним групам тварин з гіпотиреозом вводили водний екстракт ряски малої в дозі 0,5 мл/100 г маси тіла тварини та препарат порівняння Йодомарин у дозі 12 мкг йоду/кг. В результаті проведених досліджень був встановлений виразний коригуючий вплив водного екстракту листеця ряски малої на синтетичну функцію щитоподібної залози та показники вуглеводного обміну в умовах гіпотиреозу, що проявлялося в нормалізації рівня тиреоїдних гормонів, підвищенні вмісту глюкози у сироватці крові та зниженні вмісту глікогену у гомогенаті печінки. Таким чином, водний екстракт листеця ряски малої може бути віднесений до регуляторів гіпофункції щитоподібної залози та вуглеводного обміну і є перспективним для подальшого вивчення його ефективності.

Ключові слова: водний екстракт листеця ряски малої; експериментальний гіпотиреоз; перхлорат натрію; тиреоїдні гормони; глюкоза; глікоген

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ОЦЕНКА СОДЕРЖАНИЯ ГЛЮКОЗЫ И ГЛИКОГЕНА ПРИ ПРИМЕНЕНИИ ВОДНОГО ЭКСТРАКТА ЛИСТЕЦА РЯСКИ НА ФОНЕ ЭКСПЕРИМЕНТАЛЬНОГО ГИПОТИРЕОЗА

Проведены экспериментальные исследования по оценке эффективности влияния водного экстракта листеца ряски малой на показатели углеводного обмена в условиях гипотиреоидного состояния у крыс. Экспериментальный гипотиреоз у крыс вызывали введением 1 % раствора перхлората натрия вместо питьевой воды в течение 20 дней. Через 20 дней экспериментальным группам животных с гипотиреозом вводили водный экстракт ряски малой в дозе 0,5 мл/100 г массы тела животного и препарат сравнения Йодомарин в дозе 12 мкг йода/кг. В результате проведенных исследований установлено выраженное корректирующее действие водного экстракта листеца ряски малой на синтетическую функцию щитовидной железы и показатели углеводного обмена в условиях гипотиреоза, что проявлялось в нормализации уровня тиреоидных гормонов, повышении содержания глюкозы в сыворотке крови и снижении содержания гликогена в гомогенате печени. Таким образом, водный экстракт листеца ряски малой может быть отнесен к регуляторам гипофункции щитовидной железы и углеводного обмена и является перспективным для дальнейшего изучения его эффективности.

Ключевые слова: водный экстракт листеца ряски малой; экспериментальный гипотиреоз; перхлорат натрия; тиреоидные гормоны; глюкоза; гликоген

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