

UDC 616.441-008.61:582.272:615.25

## THE STUDY OF THE ANTIGOITROGENIC EFFECT OF THE EXTRACT FROM *LAMINARIA* ON THE MODEL OF MERCAZOLILUM-INDUCED HYPOTHYROIDISM

V.O.Orlova, V.M.Kravchenko, O.A.Scherbak, V.A.Georgiyants,  
I.M.Vladymyrova\*

National University of Pharmacy  
Institute for Continuing Education of Pharmacy Professionals at the National  
University of Pharmacy\*

*Key words: extract from Laminaria; thyroid gland; mercazolilum hypothyroidism; thyroid hormones*

*The paper presents the results of the experimental study of the effect of the aqueous extract from Laminaria on the thyroid function compared to Iodomarin on the model of mercazolilum-induced hypothyroidism. It has been found that the extract from Laminaria reveals the antigoitrogenic effect determined by measuring the weight of the thyroid gland, and it exceeds the effect of Iodomarin. Introduction of the extract from Laminaria and Iodomarin does not lead to recovery of the thyroid hormone level in the blood, which is significantly reduced in experimental animals with mercazolilum-induced hypothyroidism. However, taking into account the stimulating effect previously determined on the hormone-synthetic function of the thyroid gland in intact animals the marked antigoitrogenic effect revealed on the model of experimental hypothyroidism, and improvement of thyroid hormone level at the end of the experiment give grounds to conclude about the thyroid-stimulating effect of the aqueous extract from Laminaria under study.*

The most common non-infectious human diseases are those associated with iodine deficiency. According to the World Health Organization (WHO) 30% of the world's population is at risk of iodine deficiency disorders. About 700 million people have an increased thyroid gland (endemic goiter) because of iodine deficiency. Environmental deterioration also affects the prevalence of goiter epidemic. It is known that many anthropogenic environmental factors have a marked antithyroid effect, and therefore, the goitrogenic effect. The problem of iodine deficiency continues to be relevant in Ukraine [9].

Resistant and prolonged iodine deficiency is in appearance of a number of iodine deficiency disorders of the thyroid gland (diffuse and nodular goiter, hypothyroidism), miscarriage, perinatal mortality, physical and mental deficiency in children, endemic cretinism, etc.

Medical and social significance of hypothyroidism cannot be overestimated. It depends not only on its prevalence in the population and the tendency to further increase of the number of patients, but the fact that the hypofunction of the thyroid gland (TG) leads to various organ and neuropsychiatric disorders, decreased lifeware, etc. [11].

The primary method of prevention of these diseases is the use of iodine-treated salt, bread, and the main treatment is to control iodine deficiency using drugs, including herbal formulations [7].

Today in Ukraine among the registered drugs for prevention and treatment of iodine deficiency disorders are only products based on potassium iodide in the form of tablets, among them the larger market segment is occupied by drugs of foreign manufacturers, in particular Germany and the United States [2]. Therefore, development of new drugs for correction

of the thyroid hypofunction remains a pressing problem of modern medicine and pharmacy.

The search and study of herbal substances with thyroid-stimulating properties, development of dosage forms and creation of drugs on their basis are carried out in the National University of Pharmacy. The studies of the extract from *Laminaria* conducted allowed revealing the thyroid-stimulating effect in intact animals [3].

The aim of this work was to study the effect of the aqueous extract from *Laminaria Saccharina* on the thyroid function in experimental hypothyroidism.

### Materials and Methods

Manipulations with animals of the experimental vivarium were performed according to the national "General ethical principles of animal experimentation" (Ukraine, 2001), which are consistent with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) [6].

The animals were kept under standard vivarium conditions with natural light, diet recommended

V.O.Orlova – external PhD candidate of the Human Physiology and Anatomy Department of the National University of Pharmacy (Kharkiv)

I.M.Vladymyrova – Candidate of Pharmacy, assistant professor of the Department of Quality, Standardization and Certification of Medicines of the Institute for Continuing Education of Pharmacy Professionals at the National University of Pharmacy (Kharkiv)

Table 1

## Dynamics of the body weight (g) in rats during the experiment

Group	Statistical index	Day of the experiment				
		Start of the experiment	12-th	19-th	26-th	33-d
Intact control (IC)	n	6	6	6	6	6
	$\bar{x}$	118.67	156.67* <sup>‡</sup>	171.67	177.50	186.33* <sup>‡,###</sup>
	$\pm S_{\bar{x}}$	7.92	6.67	4.77	4.61	4.59
Control pathology (CP)	n	10	10	10	10	10
	$\bar{x}$	115.63	144.00* <sup>‡</sup>	171.00	177.50	161.00* <sup>‡,###</sup>
	$\pm S_{\bar{x}}$	6.30	6.27	6.00	2.83	5.42
Mercazolilum + extract from <i>Laminaria</i>	n	10	10	10	10	10
	$\bar{x}$	120.00	153.00* <sup>‡</sup>	173.50	176.88	194.00* <sup>‡,###</sup>
	$\pm S_{\bar{x}}$	8.40	7.75	6.63	8.13	6.86
Mercazolilum + Iodomarin	n	10	10	10	10	10
	$\bar{x}$	124.38	159.00* <sup>‡,###</sup>	178.50	186.25	188.00* <sup>‡,###</sup>
	$\pm S_{\bar{x}}$	6.64	5.47	5.73	6.66	5.87

## Notes:

- 1) \* – statistically significant differences compared to the beginning of the experiment;
- 2) \*\* – statistically significant differences from the group of CP;
- 3) <sup>‡</sup> p<0.05; <sup>##</sup> p<0.01; <sup>###</sup> p<0.001.

for this type of animal and drinking regimen *ad libitum*.

Thirty-six rats weighting 115-125 g were used in the experiment. Animals were randomly divided into 4 groups: group 1 – intact control (IC); group 2 – animals treated with mercazolilum (1-methyl-2-merkaptomidazol of the Pharmaceutical company "Zdorovye", Ltd., Ukraine) in the dose of 0.01 g/100 g of the body weight of the active ingredient *per os* in 10% starch for 33 days – the group of control pathology (CP) [5]; group 3 – animals treated with the extract from *Laminaria* (an aqueous residue obtained by complex processing of *Laminaria* thalli) *per os* in the dose of 1 ml/100 g of the body weight from 13 to 33 day of the experiment (21 days); group 4 – animals treated with Iodomarin 100 (Berlin-Chemie AG, Germany) in the dose of 20 µg of iodine/100 g of the body weight.

Animals were weighed on an empty stomach on the 12<sup>th</sup>, 19<sup>th</sup>, 26<sup>th</sup> and 34<sup>th</sup> days of the experiment. After 12 days the tail vein blood from the part of the experimental animals was taken for determination of serum thyroid hormones.

The animals were sacrificed from the experiment by rapid decapitation. During the autopsy the blood samples were collected in rats, thyroid gland (TG), thymus, spleen, adrenals, testes, ventral portion of the prostate were selected and weighed.

The serum concentration of thyroid hormones – thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) was detected by ELISA using commercial kits.

The statistical analysis of the results was carried out using analysis the Excel 2007 software package. The data obtained are presented as the mean ( $\bar{x}$ ), its error ( $\pm S_{\bar{x}}$ ). The null hypothesis concerning the absence of difference between groups was checked using parametric methods (using Student t-test) [4, 8]. In the case of multiple comparisons, the null hypothesis was checked using Q-criterion of Dunn [4]. Differences between groups were considered to be significant at the level of statistical significance accepted as p<0.05.

### Results and Discussion

Mercazolilum is a known thyreostatic, which mechanism of ac-

tion is in blocking the thyroid hormone synthesis on the level of interaction between mono- and diiodothyrosine and inhibiting the process of tyrosine iodination of thyroglobulin fragments. In addition, mercazolilum application leads to changes in the immune status that are evident in antithyroid antibody titre reduction, improvement of T-lymphocyte-suppressors. In the experiments previously conducted it was shown that the content of albumin and immunoglobulins of all classes in the blood decreased in animals treated with this thyreostatic [1].

In our study the weight of animals treated with mercazolilum after 12 days statistically significant increased compared to the baseline (Tab. 1). At the same time there was the weight gain in animals from the group of the intact control. At the end of the experiment (day 33) the body weight of rats from group 2 (CP) was lower than in group 1 (IC) by 13.8%. This result may be manifestation of the lack of thyroid hormones (T<sub>3</sub> and T<sub>4</sub>) in animals caused by mercazolilum. It is also well known that along with iodine-containing

Table 2

## The absolute weight of organs in rats from different groups

Indicator	Statistical index	Intact control	Control pathology	Mercazolilum + extract from <i>Laminaria</i>	Mercazolilum + Iodomarin
Thyroid gland, mg	n	6	10	10	10
	x	0.023	0.075* <sup>*,***</sup>	0.025** <sup>*,***</sup>	0.042* <sup>**,***</sup>
	±S <sub>x</sub>	0.002	0.004	0.002	0.003
Thymus, mg	n	6	10	10	10
	x	0.388	0.267	0.294	0.271
	±S <sub>x</sub>	0.040	0.019	0.020	0.020
Spleen, mg	n	6	10	10	10
	x	0.986	0.664	0.676	0.686
	±S <sub>x</sub>	0.155	0.073	0.061	0.029
Adrenal glands, mg	n	6	10	10	10
	x	0.038	0.031	0.032	0.035
	±S <sub>x</sub>	0.004	0.002	0.002	0.001
Testes, mg	n	6	10	10	10
	x	2.869	2.976	2.807	2.685
	±S <sub>x</sub>	0.085	0.104	0.041	0.185
Prostate, mg	n	6	10	10	10
	x	0.344	0.391	0.282* <sup>*,#</sup>	0.423
	±S <sub>x</sub>	0.033	0.027	0.020	0.044

## Notes:

- 1) \* – statistically significant differences from the group of IC;
- 2) \*\* – statistically significant difference from the group of CP;
- 3) # p<0.05; \* p<0.01; \*\*\* p<0.001.

thyroid hormone deficiency the growth hormone deficiency, which secretion is related to the level of thyroid hormones, develops.

Autopsy showed that the thyroid weight increased by 226.1% in animals with hypothyroidism on the 33rd day compared to the control (p<0.001), i.e. the evident goitrogenic effect of mercazolilum was observed. In addition, this group of animals had a marked decrease of the thymus and spleen weight compared to those of the intact control group. It is consistent with the literature data concerning the effects of a thyreostatic on the immune system. Other changes in the mass of the examined organs were not found (Tab. 2).

The body weight in the group of animals treated with the extract from *Laminaria* increased by 13.4% (p<0.05) at the end of the experiment compared to the control and intact groups of animals. In autopsy it was found that the weight of

the thyroid gland in rats significantly decreased (by 3 times) when compared to animals treated with mercazolilum alone and did not differ from that of the intact control, i.e. the extract from *Laminaria* prevented development of the goitrogenic effect of mercazolilum.

In addition, a slight increase in the weight of the thymus and spleen (10.1% and 1.8%, respectively) was also observed in this group of rats compared to the control pathology. It may indicate a positive tendency of the effect of the extract from *Laminaria* on this indicator. However, the decrease in the weight of prostate (27.9%) was registered. The results obtained need further studies.

The effect of the reference drug Iodomarin used to prevent thyroid disease with insufficient iodine (i.e. diffuse nontoxic goiter, diffuse euthyroid goiter) also resulted in preventing the growth of the thyroid gland weight com-

pared to the group of the experimental animals treated with mercazolilum alone. The thyroid gland weight increased by 82.6% compared to 226.1% in the control group of animals. It should be noted that this effect of Iodomarin was less prominent than the same effect of the extract from *Laminaria* (Tab. 2). At the end of the experiment the weight gain in rats treated with Iodomarin was less than in the group of animals treated with the aqueous extract from *Laminaria* under study (17.8% and 20.5%, respectively).

Changes in the thymus and spleen weight in the group of rats treated with Iodomarin were similar to those observed in rats from group 3. Only the prostate weight increased by 8.2% compared to the animals of group 2 and by 23% compared to the control group.

When measuring the concentration of iodine-containing thyroid hormones it was found that

Table 3

**The concentration of iodine-containing thyroid hormones in the blood serum of rats from different groups**

Indicator	Statistical index	Intact control	Control pathology		Mercazolilum + extract from <i>Laminaria</i>	Mercazolilum + Iodomarin
			Day 12	Day 33		
Thyroxine, nmol/L	n	6	10	10	10	10
	$\bar{x}$	57.47	32.61 <sup>*,#</sup>	25.89 <sup>*,###</sup>	35.52 <sup>*,##</sup>	31.73 <sup>*,##</sup>
	$\pm S_x$	5.45	7.12	1.97	3.12	2.49
Triiodothyronine, nmol/L	n	6	10	10	10	10
	$\bar{x}$	2.69	1.81 <sup>*,#</sup>	1.99 <sup>*,#</sup>	2.43 <sup>*,#</sup>	2.22
	$\pm S_x$	0.31	0.10	0.21	0.17	0.17
T <sub>3</sub> /T <sub>4</sub> , units	n	6	10	10	10	10
	$\bar{x}$	0.05	0.04	0.08 <sup>*,#</sup>	0.07	0.07
	$\pm S_x$	0.00	0.00	0.01	0.01	0.02

Notes:

- 1) \* – statistically significant differences from the group of IC;
- 2) \*\* – statistically significant difference from the group of CP;
- 3) <sup>\*</sup> p<0.05; <sup>\*\*</sup> p<0.01; <sup>###</sup> p<0.001.

already in 12 days of treatment with mercazolilum (simulation of experimental hypothyroidism) the level of T<sub>4</sub> and T<sub>3</sub> was significantly lower (by 43.3 and 32.7%, respectively, p<0.05) than in intact animals (Tab. 3).

At the end of the experiment (day 33) the level of hormones remained lower (T<sub>4</sub> – by 55.0%; p<0.001 and T<sub>3</sub> – 26.0%; p<0.01) compared to the intact control. The ratio T<sub>3</sub>/T<sub>4</sub> representing the character of deiodination in peripheral tissues changed from 0.05 to 0.08. It exceeds the reference values almost by 1.6 times. These data confirm correctness of the experiment in creating the model of mercazolilum-induced hypothyroidism.

The levels of thyroxin and triiodothyronine in serum increased by 37.2% (p<0.01) and 22.1% (p<0.05), respectively, in animals of group 3 compared to hypothyroidism-induced animals, but remained lower compared to the intact group of rats at the end of the experiment (day 33). The ratio of T<sub>3</sub>/T<sub>4</sub> decreased slightly from 0.08 to 0.07. The aqueous extract from *Laminaria* did not fully re-

store the synthesis of thyroid hormones in animals with mercazolilum-induced hypofunction despite its evident antioitrogenic effect.

The similar results were obtained in the group of animals treated with the reference drug Iodomarin (group 4). The concentrations of T<sub>4</sub> and T<sub>3</sub> increased by 22.6% (p<0.01) and 11.6%, respectively. Therefore, Iodomarin was inferior to the extract from *Laminaria* by the specific effect on the thyroid gland.

This effect can be explained by short-term introduction of the extract from *Laminaria* studied and the reference drug Iodomarin, dose and the mechanism of experimental hypothyroidism development. It was determined that mercazolilum revealed a strong inhibiting action on the biosynthesis of thyroid hormones (group 2 of the experimental animals). Probably the extract from *Laminaria* as a prophylactic agent would prevent development of the iodine deficiency state and promote earlier restoration of the hormone-synthetic function of the gland. Elucidation of the mechanisms of the thyroid-

stimulating effect on the thyroid gland requires further research.

#### CONCLUSIONS

1. On the model of mercazolilum-induced hypothyroidism the extract from *Laminaria* in the dose of 1 ml/100 g of the body weight shows the antioitrogenic effect (prevention of the thyroid weight increase), which exceeds the effect of the reference drug Iodomarin.

2. The extract from *Laminaria* and Iodomarin in our experiments does not show complete recovery of the thyroid hormone production function (by the level of thyroid hormones in the blood serum) in rats with mercazolilum-induced hypothyroidism compared to intact animals. However, increase of the level of thyroxin and triiodothyronine is observed in animals treated with extract from *Laminaria* by 37.2% and 22.1%, respectively. When using Iodomarin the increased concentrations of T<sub>4</sub> by 22.6% and T<sub>3</sub> – by 11.6% were observed.

3. The thyroid-stimulating effect of the aqueous extract from *Laminaria* studied exceeds the effect of the reference drug Iodomarin.

## REFERENCES

1. Бондарь Т.Н. // Вісник Харківського національного університету ім. В.Н.Каразіна. Сер. Біологія. – 2009. – №878, вип. 10. – С. 102-108.
2. Владимірова І.М., Георгіянци В.А. // Фармац. часопис. – 2010. – №4. – С. 90-93.
3. Владимірова І.М., Кравченко В.М., Георгіянци В.А. // Клінічна фармація. – 2011. – Т. 15, №3. – С. 67-69.
4. Гланц С.А. Медико-биологическая статистика. – М.: Практика, 1998. – 459 с.
5. Доклінічні дослідження лікарських засобів: Метод. рекомендації. / За ред. О.В.Стефанова. – К.: Авіценна, 2001. – 528 с.
6. Загальні етичні принципи експериментів на тваринах // Ендокринологія. – 2003. – Т. 8, №1. – С. 142-145.
7. Кваченюк А.Н., Кваченюк Е.Л. // Врачебное дело. – 2012. – №3-4. – С. 1-4.
8. Лакин Г.Ф. Биометрия: Учеб. пособие для биол. спец. вузов. – М.: Высш. шк., 1990. – 352 с.
9. Проблема йододифіциту в Україні [Електронний ресурс] – Режим доступу: <http://medstrana.com/articles/300/>
10. Constantinou K., Marigoula M., Valcana T. // Molecular and Cellular Biochemistry. – 2005. – Vol. 278. – P. 93-1005.
11. Ordooei M., Mottaghipisheh H., Fallah R., Rabiee A. // Iran J. Child Neurol. – 2014. – Vol. 8 (4). – P. 28-32.

**ДОСЛІДЖЕННЯ АНТИЗОБОГЕННОГО ЕФЕКТУ ЕКСТРАКТУ ЛАМІНАРІЇ НА МОДЕЛІ МЕРКАЗОЛІЛОВОГО ГІПОТИРЕОЗУ**

**В.О.Орлова, В.М.Кравченко, О.А.Щербак, В.А.Георгіянци, І.М.Владимірова\***

**Національний фармацевтичний університет, Інститут підвищення кваліфікації спеціалістів фармації  
Національного фармацевтичного університету\***

*Ключові слова:* екстракт ламінарії; щитоподібна залоза; мерказоліловий гіпотиреоз; тиреоїдні гормони

*Представлені результати експериментального вивчення впливу водного екстракту ламінарії на функцію щитоподібної залози в порівнянні з йодомарином на моделі мерказолілового гіпотиреозу. Встановлено, що екстракт ламінарії виявляє антизобогенний ефект, визначений за результатами вимірювання маси щитоподібної залози, який перевершує ефект йодомарину. Введення екстракту ламінарії і йодомарину не приводить до відновлення рівня тиреоїдних гормонів у крові, який значно знижується на тлі експериментального гіпотиреозу. Але, враховуючи раніше встановлений стимулювальний вплив на гормоносинтезуючу функцію щитоподібної залози в інтактних тварин, виявлений виразний антизобогенний ефект на моделі експериментального гіпотиреозу та визначене підвищення рівня тиреоїдних гормонів у кінці експерименту дають підставу зробити висновок про тиреоїдстимулювальний ефект досліджуваного водного екстракту ламінарії.*

**ИССЛЕДОВАНИЕ АНТИЗОБОГЕННОГО ЭФФЕКТА ЭКСТРАКТА ЛАМИНАРИИ НА МОДЕЛИ МЕРКАЗОЛИЛОВОГО ГИПОТИРЕОЗА**

**В.А.Орлова, В.Н.Кравченко, Е.А.Щербак, В.А.Георгіянци, И.Н.Владимірова\***

**Национальный фармацевтический университет, Институт повышения квалификации специалистов  
фармации Национального фармацевтического университета\***

*Ключевые слова:* экстракт ламинарии; щитовидная железа; мерказолиловый гипотиреоз; тиреоидные гормоны

*Представлены результаты экспериментального изучения влияния водного экстракта ламинарии на функцию щитовидной железы в сравнении с йодомарином на модели мерказолилового гипотиреоза. Установлено, что экстракт ламинарии имеет антизобогенный эффект, определенный по результатам измерения массы щитовидной железы, который превышает эффект йодомарина. Введение экстракта ламинарии и йодомарина не приводит к восстановлению уровня тиреоидных гормонов в крови, который значительно снижается при экспериментальном гипотиреозе. Но, учитывая ранее установленное стимулирующее влияние на гормонсинтетическую функцию щитовидной железы у интактных животных, выявленный выраженный антизобогенный эффект на модели экспериментального гипотиреоза и определенное нами повышение уровня тиреоидных гормонов в конце эксперимента дают основание сделать вывод о тиреоидстимулирующем действии исследуемого водного экстракта ламинарии.*

Address for correspondence:

12, Kulykivska str., Kharkiv, 61002, Ukraine.

Tel. (57) 706-30-73. E-mail: [scherbak\\_al@bk.ru](mailto:scherbak_al@bk.ru).

National University of Pharmacy

Received in 12.07.2016