HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITY OF ARGinine CONTAINING BEARBERRY LEAVES EXTRACT IN INSULIN RESISTANT RATS

Introduction. In recent decades, diabetes mellitus type 2 (DM2) has become one of the leading causes of deaths worldwide. A number of studies confirmed the causal relationship between the development of insulin resistance (IR) and DM2. At the same time, traditionally and for many years the plants or substances isolated from them have been using in the DM2 treatment and correction of its complications.

The aim of the study – to find out the effect of ethanolic polyphenol Bearberry leaves (Arctostaphylos uva-ursi) extract enriched with arginine (PE50_arg) on tolerance to glucose and lipid metabolism under experimental IR in rats.

Research Methods. Adult male outbred albino rats were used in the present study. Two experimental IR models were conducted: daily intraperitoneal administration of dexamethasone and a diet enriched with fructose. Treating was performed by oral administration of polyphenolic alcohol extract (PE50) and the corresponding extract with the addition of arginine (PE50_arg). IR was confirmed by measuring immunoreactive insulin (IRI) and plasma glucose levels. At the end of the experiment, the lipid profile was investigated in the obtained serum samples. The statistical processing of the data was carried out using the STATISTICA program (StatSoft Inc., USA, version 6.0).

Results and Discussion. A diet for 7 weeks enriched with fructose caused IR in rats. Also we observed increased triacylglycerol (TAG), free fatty acids (FFA) and cholesterol (Ch) levels. Daily injections of dexamethasone, which maintained the hormone level for 5 weeks, led to the IR development. Under hormone-induced IR also FFA and TAG levels were elevated, but Ch concentration in blood plasma did not significantly change. Both extracts, PE50 and PE50_arg, improve cell sensitivity to insulin in experimental IR models. At the same time, PE50_arg has a more pronounced normalizing effect on the lipid parameters being investigated.

Conclusions. Our results suggest that PE50_arg may be a potentially promising anti-diabetic agent.

KEY WORDS: insulin resistance; diabetes mellitus type 2; bearberry; arginine; hypoglycemic action.
insulin-sensitivity, as a result, it stimulates the glucose flow into cells, as well as its metabolic transformations, developed Bearberry leaves extract was enriched with arginine [9]. All the technological procedures were performed at the National University of Pharmacy (NUPh) Pharmacognosy Department by the supervising of Prof. О. M. Ko-shovyi.

The aim of the study. The present study was conducted to find out the effect of ethanolic polyphenol Bearberry leaves (Arctostaphylos uva-ursi) extract enriched with arginine (PE50_arg) on tolerance to glucose and lipid metabolism under experimental IR in rats.

RESEARCH METHODS. Adult male outbred albino rats weighing 175–200 g were used in the present study. Animals were obtained from and housed in vivarium of the NUPh Central Scientific-Research Laboratory. For experimental IR induction in rats were used two different models. Glucocorticoid-induced IR was developed by daily intraperitoneally administration of dexamethasone (15 mkg/kg/day) for 5 weeks (Dex) [10]. Diet-induced IR was caused by “watering” with 20 % fructose water solution (with free access) during 7 weeks – high-fructose diet (HFD). The animals of the intact control and the dexamethasone groups had free access to tap water. All the groups were fed with rat-food pellet ad libitum. Animals, which were randomized to group treatment, were given polyphenol ethanolic extract (extractor – 50 % ethanol, PE50) and PE50_arg beginning from the 5th week of the experiment for 2 weeks in dose 100 mg/kg b.w. Intact control group received the same volume of physiological solution. As reference preparation was used Arphasetin infusion in dose 18 ml/kg b.w. Intact control group received the same volume of physiological solution. As reference preparation was used Arphasetin infusion in dose 18 ml/kg b.w. Thereby, the data obtained indicate a decrease in the cell insulin sensitivity and the IR development. It is known that the state of hyperglycemia and IR is also accompanied by the oxidative stress development, increased lipid peroxidation and lipid metabolism disorders [11].

Table 1 – Fasting blood plasma glucose, immunoreactive insulin and HOMA indices after 5th and 7th week of the experiment

| Groups/indices | Time | FBG mmol/l | IRI pmol/l | HOMA |
|---------------|------|------------|------------|------|
| HFD | initial | 4.17±0.65 | 72±7 | 1.92±0.12 |
| | 5th week | 6.27±0.79* | 97±8* | 3.89±0.29* |
| | 7th week | 7.01±0.93* | 109±11* | 4.86±0.31* |
| | 7th week / treatment | PE50 | PE50_arg | Arf |
| | FBG mmol/l | 5.57±0.56* | 5.38±0.49* | 5.53±0.46* |
| | IRI pmol/l | 78±5* | 77±9* | 87±7* |
| | HOMA | (N<3,0) | (N<3,0) | (N<3,0) |
| Dex | 4.03±0.31 | 6.97±0.57* | 7.34±0.51* | 5.98±0.49* | 5.54±0.27 | 5.87±0.65* |
| | 101±9* | 117±11* | 79±9 | 80±9 | 87±7 |
| | 4.51±0.97* | 5.50±1.11* | 2.89±0.67* | 2.84±0.34 | 3.27±0.45* |

Note. Data were expressed as mean±SD.
* – p≤0.05 vs. intact group; ** – p≤0.05 vs. IR_HFD group and IR_Dex group respectively.

RESULTS AND DISCUSSION. Performing research tasks, it was found that keeping animals on HFD was accompanied by an increase in glucose level by 50 % and 68 % to the end of 5th and 7th weeks of the experiment, respectively. To evaluate the IR development the insulin content in blood plasma was also determined (Table 1). Recalculation showed that the HOMA index was significantly increased in the control periods. Thus, the data obtained indicate a decrease in the cell insulin sensitivity and the IR development. It is known that the state of hyperglycemia and IR is also accompanied by the oxidative stress development, increased lipid peroxidation and lipid metabolism disorders [11].
The effectiveness of different modifications of diet enriched with fructose, which lead to IR and DM2 development, was proved by experiments and explained by theory. It is known that fructose metabolism differs from glucose conversion and occurs along a pathway that is independent of insulin. Fructose in the liver under the influence of fructokinase is converted to fructose-1-phosphate, which, with the participation of aldolase, is broken down to glyceraldehyde and dihydroxyacetone phosphate. The obtained products are included in the process of gluconeogenesis, as well as converted to acetyl-CoA, followed by inclusion in lipogenesis [12]. Strengthening gluconeogenesis, which is not controlled by insulin, makes the main contribution to the development of hyperglycemia (Table 1). Moreover, there is literature data that in rats kept on a diet with a high level of fructose, it reduces the expression of insulin receptor substrate 1 (IRS-1) and phosphatidylinositol 3-kinase (PI3K) to insulin and the development of IR [13]. Acetyl-CoA overproduction can lead to hypertriglyceridemia and hypercholesterolemia development, which are observed in our experiment (Table 2). The PE50_arg introduction to HFD group animals during 2 weeks led to a significant decrease in blood glucose (Table 1). The PE50_arg introduction inhibited the development of hyperglycemia, reduced insulin levels (Table 1), and normalized the content of neutral lipids in the blood in animals kept on HFD (Table 2).

Dexamethasone-induced IR is also well-established model, but has another metabolic mechanism. The results of the dexamethasone injection after 5 weeks showed that the blood glucose level of animals in this group by 82% was higher than the values of the intact group (Table 1). It was found out that the insulin signal implementation in target tissues is carried out with the participation of IRS, PI3K, which activates the protein kinase B (Akt). Akt-serine/threonine kinase, in turn, activates the translocation of glucose transporter type 4 (glut-4) and stimulates the glucose uptake into the cell. The dexamethasone administration is accompanied by inhibition of Akt insulin-dependent phosphorylation, which leads to glucose uptake inhibition and the IR development. It is known that glucocorticoids stimulate fatty acids and TAG synthesis in the liver, as a result, elevated level of these lipids in the blood [14]. The data obtained in our work confirm this thesis (Table 2); at the same time the Ch content under the dexamethasone action did not significantly change. As for hypoglycemic action, the PE50_arg administration during 14 days led to a significant decrease in the glucose level (Table 1) more pronounced compared with PE50 administration. A significant decrease in the HOMA index was also observed.

The results of this study demonstrated that arginine addition to the polyphenol preparation improved its hypoglycemic action.

The arginine administration is known to stimulate the expression of PI3K and Akt which entails an increase in the sensitivity of cells to insulin [15]. This evidence found the confirmation in our current experiment. It is known that a prolonged increase in FFA content in the blood is an important manifestation of IR development, which mediated by endoplasmic reticulum stress and activation of c-Jun N-terminal kinases (Jnk) [16]. The decrease in the content of FFA observed in our work (Table 2) leads to an increase in the target cells sensitivity to the insulin action. Most likely it was caused by improving of FFA oxidation in the liver by the arginine action [17]. Also, it is quite probable explanation for the cholesterol content decrease in blood serum (Table 2).

Thus, the obtained results indicate that prolonged HFD and dexamethasone administration for 7 weeks contributed to the development of carbohydrate metabolism disorders (glucose resistance and hyperglycemia), which may serve as criteria for IR development. The 2-weeks PE50 and PE50_arg administration under IR led to improvement of these disorders.

Table 2 – The effect of PE50 and PE50_arg on the content of individual lipid fractions in the blood serum of rats with IR

|       | TL mg/g tissue | TAG mg/g tissue | FFA mmol/g tissue | Ch mmol/g tissue |
|-------|----------------|----------------|------------------|-----------------|
| IC    | 171±7          | 6.17±0.56      | 4.13±0.57        | 17.7±2.05       |
| IR_HFD | 229±13*        | 10.29±1.21*    | 6.11±0.45*       | 25.6±2.62*      |
| IR_HFD_PE50 | 264±27**     | 7.42±0.67**    | 5.43±0.41**      | 21.9±1.71       |
| IR_HFD_PE50_arg | 249±19**     | 6.57±0.87**    | 4.65±0.37**      | 17.8±1.82       |
| IR_Dex | 273±15*        | 11.97±1.19     | 7.18±0.24*       | 19.3±1.21       |
| IR_Dex_PE50 | 251±18**     | 8.14±0.97      | 6.39±0.41**      | 18.4±1.14       |
| IR_Dex_PE50_arg | 231±17**     | 6.57±0.67      | 4.58±0.63**      | 19.4±2.3        |

Note. Data were expressed as mean±SD.  
* – ps0.05 vs. intact group; ** – ps0.05 vs. IR_HFD group and IR_Dex group respectively.

ISSN 2410-681X. Медична та клінічна хімія. 2020. Т. 22. № 1
CONCLUSIONS. 1. High-fructose diet during 7 weeks induced insulin resistance in rats.
2. Dexamethasone daily injections that maintained the hormone level during 5 weeks led to the insulin resistance development.

3. Both PE50 and PE50_arg improve insulin cell sensitivity under experimental IR models. At the same time PE50_arg has more pronounced normalizing effect on studied lipid indices.
4. Our results suggest that PE50_arg may be a potentially promising anti-diabetic agent.

LIST OF LITERATURE

1. Epidemiology of type 2 diabetes / M. A. B. Khan, M. J. Hashim, J. K. King [et al.] // Global Burden of Disease and Forecasted Trends Journal of Epidemiology and Global Health. – 2020. – 10, No. 1. – P. 107–111.
2. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045 / N. H. Cho, J. E. Shaw, S. Karuranga [et al.] // Diabetes Research and Clinical Practice. – 2019. – 138. – P. 271–281.
3. Insulin resistance: Review of the underlying molecular mechanisms / H. Yaribeygi, F. R. Farrokhi, A. E. Butler [et al.] // Journal of Cellular Physiology. – 2019. – 234, No. 6. – P. 8152–8161.
4. Petersen M. C. Mechanisms of insulin action and insulin resistance / M. C. Petersen, G. I. Shulman // Physiological Reviews. – 2018. – 98, No. 4. – P. 2133–2223.
5. Bjornstad P. Pathogenesis of lipid disorders in insulin resistance: A brief review / P. Bjornstad, R. H. Eckel // Current Diabetic Reports. – 2018. – 23, No. 12. – P. 127.
6. Spiller S. Plasma levels of free fatty acids correlate with type 2 diabetes mellitus diabetes, obesity and metabolism / S. Spiller, M. Blüher, R. Hoffmann // A Journal of Pharmacology and Therapeutics. – 2018. – 20, No. 11. – P. 2661–2669.
7. Al-Snafi A. E. Medicinal plants with antidiabetic effects – an overview / A. E. Al-Snafi, W. J. Majid, T. A. Talab // IOSR Journal of Pharmacy. – 2019. – 14, No. 3. – P. 9–46.
8. Kravchenko G. Screening of Bearberry leaves extracts hypoglycemic effect and study of acute toxicity / G. Kravchenko, M. Mazen, O. Krasilnikova // Укра. біофармац. журн. – 2018. – 55, No. 2. – P. 13–16.
9. Hu S. L-arginine modulates glucose and lipid metabolism in obesity and diabetes / S. Hu, M. Han, A. Rezaei [et al.] // Current Protein & Peptide Science. – 2017. – 18, No. 6. – P. 599–608.

REFERENCES

1. Khan, M.A.B., Hashim, M.J., King, J., Govender, R.D., Mustafa, H., & Al Kaabi, J. (2020). Epidemiology of type 2 diabetes. Global Burden of Disease and Forecasted Trends Journal of Epidemiology and Global Health, 10, 107-111.
2. Cho, N.H., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohrrogge, A.W., & Malanda, B. (2019). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Research and Clinical Practice, 138, 271-281.
3. Yaribeygi, H., Farrokhi, F.R., Butler, A.E., & Sahebkar, A. (2019) Insulin resistance: Review of the underlying molecular mechanisms. Journal of Cellular Physiology, 234, 8152-8161.
4. Petersen, M.C., & Shulman, G.I. (2018). Mechanisms of insulin action and insulin resistance. Physiological Reviews, 98, 2133-2223.
5. Bjornstad, P., & Eckel, R.H. (2018). Pathogenesis of lipid disorders in insulin resistance: A brief review. Current Diabetic Reports, 18, 127.

10. Модифікація методу моделювання експериментальної інсулінорезистентності у щурів: іформаційний лист Укрмультпатентінформу про нововведення в системі Охорони здоров'я № 86-2015 / А. Л. Загайко, Т. О. Брюханова, А. і. Шкапо. – К., 2015. – 3 с.
11. Вивчення гепатопротекторної активності рослинних поліфенолів на моделі експериментальної інсулінорезистентності / А. Л. Загайко, О. А. Красильникова, Г. Б. Кравченко, Ю. І. Кочубей // Світ медицини та біології. – 2017. – 59, No. 1. – С. 117–121.
12. Tran L. T. The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension / L. T. Tran, V. G. Yuen, J. H. McNeill // Molecular and Cellular Biochemistry. – 2009. – 332, No. 1–2. – P. 145–159.
13. Czech M. P. Insulin action and resistance in obesity and type 2 diabetes / M. P. Czech // Nature Medicine. – 2017. – 23, No. 7. – P. 804–814.
14. Geer E. B. Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism / E. B. Geer, J. Islam, C. Buettner // Endocrinology and Metabolism Clinics of North America. – 2014. – 43, No. 1. – P. 75–102.
15. Effect of L-arginine supplementation on the hepatic phospholipidinositol 3 kinase signaling pathway and glucoseogenic enzymes in early intrauterine growth-restricted rats / K. Luo, P. Chen, S. Li [et al.] // Experimental and Therapeutic Medicine. – 2017. – 14. – P. 2355–2360.
16. Sears B. The role of fatty acids in insulin resistance / B. Sears, M. Perry // Lipids in Health and Disease. – 2015. – 14. – P. 121.
17. Regulatory roles for L-arginine in reducing white adipose tissue / B. Tan, X. Li, Y. Yin [et al.] // Frontiers in Bioscience. – 2012. – 17. – P. 2237–2246.
6. Spiller, S., Blüher, M., & Hoffmann, R. (2018) Plasma levels of free fatty acids correlate with type 2 diabetes mellitus diabetes, obesity and metabolism. A Journal of Pharmacology and Therapeutics, 20, 2661-2669.
7. Al-Snafi, A.E., Majid, W.J., & Talab, T.A. (2019) Medicinal plants with antiabetic effects – an overview. IOSR Journal of Pharmacy, 9, 9-46.
8. Kravchenko, G., Mazen, M., & Krasilnikova, O. (2018). Screening of Bearberry leaves extracts hypoglycemic effect and study of acute toxicity. Ukrainskyi Biofarmacevtichnyi Journal, 2, 13-16.
9. Hu, S., Han, M., Rezaei, A., Li, D., Wu, G., & Ma, X. (2017). L-arginine modulates glucose and lipid metabolism in obesity and diabetes. Current Protein & Peptide Science, 18, 599-608.
10. Zahaiko, A.L., Briukhanova, T.O., & Shkapo, A.I. (2015). Modyfikatsiia metodu modeliuvannia eksperimentalnoi insulinorezystentnosti u shchuriv: Informatsiiniy lyst Ukrmedpatentinformu [Modification of the experimental insulin resistance simulation method in rats: Ukrmedpatent Information Sheet on Innovation in Healthcare System No. 86-2015]. No. 86-2015, Kyiv [in Ukrainian].
11. Zagayko, A.L., Krasilnikova, O.A., Kravchenko, G.B., & Kochubei, Y.I. (2017). Vyvchennia hepatoprotetkornoi aktyvnosti roslin polіfenolіv na modeli eksperymentalnoi insulinorezystentnosti [Study of hepato-protective activity of plant polyphenols on a model of experimental insulin resistance]. Svit biolohii ta medytysny – World of Biology and Medicine, 59, 117-121 [in Ukrainian].
12. Tran, L.T. Yuen, V.G., & McNeil, J.H. (2009). The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Molecular and Cellular Biochemistry, 332, 145-59.
13. Czech, M.P. (2017 Insulin action and resistance in obesity and type 2 diabetes. Nature Medicine, 23, 804-814.
14. Geer, E.B., Islam, J., & Buettner, C. (2014). Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism. Endocrinology and Metabolism Clinics of North America, 43, 75-102.
15. Luo, K., Chen, P., Li, S., Li, W., He, M., Wang, T., & Chen, J. (2017). Effect of L-arginine supplementation on the hepatic phosphatidylinositol 3 kinase signaling pathway and gluconeogenic enzymes in early intrauterine growth-restricted rats. Experimental and Therapeutic Medicine, 14, 2355-2360.
16. Sears, B., Perry, M. (2015) The role of fatty acids in insulin resistance. Lipids in Health and Disease, 14, 121.
17. Tan, B., Li, X., Yin, Y., Wu, Z., Liu, C., Tekwe, C.D., Wu G. (2012) Regulatory roles for L-arginine in reducing white adipose tissue. Frontiers in Bioscience, 17, 2237-2246.
та поліфенольний спиртовий екстракт з додаванням аргініну покращували чутливість клітин до інсуліну в експериментальних іР-моделях. Водночас поліфенольний спиртовий екстракт з додаванням аргініну мав більш виражену нормалізуючу дію на досліджувані піпідні показники.

**Висновок.** Результати нашого експерименту свідчать про те, що аргінін може бути потенційно перспективним протидіабетичним засобом.

**КЛЮЧОВІ СЛОВА:** інсулінорезистентність; цукровий діабет 2 типу; мучниця звичайна; аргінін; гіпоглікемічна дія.

---

**ГІПОГЛІКЕМІЧЕСКАЯ И ГИПОЛИПИДЕМИЧЕСКАЯ АКТИВНОСТЬ ЭКСТРАКАТА ИЗ ЛИСТЬЕВ ТОЛОКНЯНКИ ОБЫКНОВЕННОЙ С ДОБАВЛЕНИЕМ АРГІНІНА НА ФОНЕ ИНСУЛІНОРЕЗИСТЕНТНОСТИ У КРЫС**

Резюме

Вступление. В последние десятилетия сахарный диабет 2 типа стал одной из ведущих причин смертности во всем мире. Результаты ряда исследований подтвердили причинно-следственную связь между развитием инсулинорезистентности (ИР) и этим заболеванием. В то же время традиционно и в течение многих лет растения или вещества, выделенные из них, используют для лечения сахарного диабета 2 типа и коррекции его осложнений.

Цель исследования – выяснить влияние спиртового полифенольного экстракта из листьев толокнянки обыкновенной, который обогащен аргинином, на толерантность к глюкозе и метаболизм липидов при экспериментальной инсулинорезистентности у крыс.

Методы исследования. Эксперимент проведен на взрослых крысах-альбиносах. Были использованы различные модели инсулинорезистентности, такие, как ежедневное внутрибрюшное введение дексаметазона и диета, обогащенная фруктозой. Для коррекции перорально вводили полифенольный спиртовой экстракт и полифенольный спиртовой экстракт с добавлением аргинина. Инсулинорезистентность подтверждали путем измерения уровня иммунореактивного инсулина и уровня глюкозы в плазме крови. По окончании эксперимента в отобранных образцах сыворотки крови определяли показатели липидного профиля. Статистическую обработку данных осуществляли с помощью программы STATISTICA (StatSoftInc. США, версия 6.0).

Результаты и обсуждение. Диета с высоким содержанием фруктозы в течение 7-ми недель вызывала резистентность к инсуліну у крыс. Также наблюдали увеличенный уровень триацилглицеролов, свободных жирных кислот и холестерола. Ежедневные инъекции дексаметазона, которые поддерживали уровень гормона в течение 5-ти недель, обусловили развитие резистентности к инсулину. При инсулинорезистентности, которая была индуцирована гормонами, уровень свободных жирных кислот и триацилглицеролов также был повышен, однако концентрация в плазме крови холестерола достоверно не изменялась. Полифенольный спиртовой экстракт с добавлением аргинина улучшил чувствительность клеток к инсулину в экспериментальных ИР-моделях. В то же время полифенольный спиртовой экстракт имел более выраженное нормализующее действие на исследуемые липидные показатели.

Вывод. Результаты нашего эксперимента свидетельствуют о том, что полифенольный спиртовой экстракт может быть потенциально перспективным противodiабетическим средством.

**КЛЮЧЕВЫЕ СЛОВА:** инсулиновая резистентность; сахарный диабет 2 типа; толокнянка обыкновенная; аргинин; гипогликемическое действие.

Address for correspondence: G. B. Kravchenko, National University of Pharmacy, Pushkinska street 53, Kharkiv, 61002, Ukraine, e-mail: annabk2014@gmail.com.

Received 27.01.20