THE STUDY OF THE HYPOGLYCEMIC ACTION OF EXTRACTS FROM BEARBERRY LEAVES UNDER EXPERIMENTAL INSULIN RESISTANCE IN RATS

Type 2 diabetes mellitus (DM2) has been increasing steadily all over the world. A large number of medicinal plants that have the hypoglycemic effect are known, but, unfortunately, currently there is very limited choice of antidiabetic herbal medicines.

Aim. To design the experiment in order to study the hypoglycemic effect of polyphenolic extracts from bearberry (Arctostaphylos uva-ursi) leaves under the experimental insulin resistance (IR) in rats.

Materials and methods. The experimental IR was induced by dexamethasone injections (Dex) and feeding with a high-fructose diet (HFD). Male outbred albino rats were randomized depending on the purpose of the experiment. As the study objects 50 % ethanolic polyphenol extracts obtained from bearberry leaves with addition of arginine (PE50_arg) and cysteine (PE50_cys) were selected. The oral glucose tolerance test (OGTT) was performed in all experimental groups of animals.

Results. Dex had a more pronounced effect on tolerance to glucose compared to the HFD. It was shown that PE50_arg and PE50_cys after two weeks of administration revealed the ability to decrease the blood glucose level in rats, as well as reduce IR development and improve tolerance to glucose under the experimental IR. The hypoglycemic activity found did not much differ from the action of Metformin, but exceeded the activity of Arphazetin. These results can be the evidence of activation of glucose utilization processes, and it, in turn, indicates the insulin sensitivity improvement due to the action of the extracts studied. The data obtained indicate that the corrective effect of arginine and cysteine on signal transduction processes in insulin target cells plays an important role in the IR treatment.

Conclusions. Bearberry leaves are the promising raw material for creating an anti-diabetic drug. Thus, it is necessary to further study the mechanisms of regulation of metabolic disorders when introducing new polyphenolic extracts.

Key words: insulin resistance; bearberry; oral glucose tolerance test; diabetes mellitus type 2; hypoglycemic action
Исследование гипогликемического действия экстрактов, полученных из листьев толокнянки обыкновенной, при экспериментальной инсулинорезистентности у крыс

Заболеваемость сахарным диабетом 2-го типа (СД2) постоянно возрастает во всем мире. Было найдено большое количество лекарственных растений, которые имеют гипогликемическое действие, но, к сожалению, на сегодняшний день существует очень ограниченный выбор растительных противодиабетических лекарственных препаратов.

Цель исследования. Этот эксперимент был разработан для изучения гипогликемического эффекта полифенольных экстрактов из листьев Толокнянки обыкновенной (Arctostaphylos uva-ursi) при экспериментальной инсулинорезистентности (ИР) у крыс.

Материалы и методы. Экспериментальную ИР вызывали инъекциями дексаметазона (Dex) и диетой с высоким содержанием фруктозы (HFD). Самцы крыс-альбиносов были распределены по группам в зависимости от цели эксперимента. В качестве объектов исследования были отобраны 50 % этанольные полифенольные экстракты, полученные из листьев Толокнянки обыкновенной с добавлением аргинина (PE50_arg) и цистеина (PE50_cys). Пероральный тест на толерантность к глюкозе (ОГТТ) проводили во всех экспериментальных группах животных.

Результаты. Инъекции дексаметазона имели более выраженное влияние на толерантность к глюкозе по сравнению с HFD. Было показано, что PE50_arg и PE50_cys после двух недель введения проявили способность снижать уровень глюкозы в крови крыс, ослаблять развитие ИР и снижать толерантность к глюкозе по экспериментальной ИР. Обнаруженная гипогликемическая активность мало отличалась от действия метформина, но превышала активность арфазетина. Эти результаты могут быть свидетельством активации процессов утилизации глюкозы, что, в свою очередь, указывает на улучшение чувствительности к инсулину благодаря действию экстрактов. Полученные данные свидетельствуют о том, что корректирующее воздействие аргинина и цистеина на процессы передачи сигнала в клетках-мишених инсулина играет важную роль в лечении ИР.

Выводы. Листья Толокнянки обыкновенной являются многообещающим сырьем для создания антидиабетического лекарственного препарата. Таким образом, необходимо дальнейшее изучение механизмов регуляции метаболических нарушений при введении новых полифенольных экстрактов.

Ключевые слова: инсулинорезистентность, толокнянка обыкновенная; пероральный тест на толерантность к глюкозе; сахарный диабет 2-го типа; гипогликемическое действие
Glycemic variation and oral glucose tolerance test under the experimental insulin resistance compared to administration of pure extract (PE50), arginine (PE50_arg) and cysteine (PE50_cys) in healthy animals

| Groups          | Blood glucose level (mmol/l) at various time intervals |
|-----------------|--------------------------------------------------------|
|                 | 0 min | 30 min | 60 min | 90 min | 120 min |
| Intact control  | 4.07±0.35 | 5.84±0.64 | 7.21±0.85 | 5.08±0.79 | 3.78±0.56 |
| PE50            | 4.03±0.54 | 5.47±0.65 | 7.07±0.77 | 6.42±0.71 | 4.59±0.49 |
| PE50_arg        | 4.05±0.66 | 5.35±0.58 | 6.81±0.74 | 5.78±0.67 | 4.47±0.51 |
| PE50_cys        | 4.04±0.57 | 5.51±0.63 | 7.03±0.69 | 5.93±0.72 | 4.57±0.53 |
| HFD 30 min      | 7.12±0.64 \(^a\) | 10.61±0.71 \(^a\) | 11.88±0.98 \(^a\) | 10.11±0.84 \(^a\) | 8.61±0.83 \(^a\) |
| HFD 60 min      | 9.01±0.59 \(^a\) | 17.20±0.81 \(^a\) | 19.87±0.93 \(^a\) | 15.49±0.75 \(^a\) | 12.21±0.63 \(^a\) |

Note. Values are expressed as mean ± SEM from 6 rats; \(^a\) p<0.05 vs IC group.

7 weeks – high-fructose diet (HFD) [6]. As the study objects 50 % ethanolic polyphenol extracts obtained from bearberry leaves with addition of arginine (PE50_arg) and cysteine (PE50_cys) and a pure extract (PE50) in the dose of 100 mg/kg b.w. were selected [7]. All these substances were developed at the Pharmacognosy Department of the NUPh under the supervision of professor Koshevoy O. M. As the reference drugs Arphasetin infusion (arph) in the recommended dose recalculated for rats (18 ml/kg b.w.) and Metformin (met) in the dose of 100 mg/kg b.w. were selected [8].

Animals were randomly divided into 16 experimental groups (n = 6 rats) as follows: 1 – healthy animals, intact control (IC); 2-4 – healthy animals taken PE50, PE50_arg, PE50_cys, respectively, (groups specified as PE50, PE50_arg, PE50_cys); 5 – animals with IR induced by HFD (specified as HFD); 6 – animals with IR induced by dexamethasone injection (specified as Dex); 7-11 – animals fed with HFD and beginning from the 5\(^{th}\) week of the experiment administered PE50, PE50_arg, PE50_cys, arph and met, respectively, for 2 weeks (groups specified as HFD-PE50, HFD-PE50_arg, HFD-PE50_cys, HFD-arph, HFD_met); 12-16 – animals injected with Dex and beginning from the 5\(^{th}\) week of the experiment administered PE50, PE50_arg, PE50_cys, arph and met, respectively, for 2 weeks (specified as Dex PE50, Dex PE50_arg, Dex PE50_cys, Dex arph, Dex met).

The oral glucose tolerance test (OGTT) was performed in all experimental groups of animals. When conducting OGTT the blood samples were taken in 0, 30, 60, 90 and 120 min after oral administration of glucose solution (3 g/kg b.w.) via gastric tube [9]. Blood samples were obtained by gingival vein punction [10]. The blood glucose (BG) concentration was determined using a “One Touch Select” glucometer (LifeScan, USA). The areas under the curves (AUC) were calculated using the trapezoidal rule [11]. The IR development was confirmed by measuring the immunoreactive insulin level and the fasting blood plasma glucose level in 5 weeks and 7 weeks of the experiment [6].

All manipulations were performed according to the “Protocol of Amendment to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986, as amended, 1998), the Law of Ukraine “On protection from cruelty to animals” (dated 15.12.2009, No. 1759-VI), and the European Union Directives 2010/10/63 EU about animal experiments. All results were expressed as mean ± SEM (standard error of mean). The data were analyzed using STATISTICA 6. The value of p<0.05 was considered significant.

Results and discussion

The HFD and Dex groups of rats showed a significant BG level elevation in fasting state compared to normal animals (Table). The OGTT conducted confirmed the IR development in HFD group of animals since we observed a considerable increase in glucose levels not only on the 30th, 60th and 90th min of the experiment, but even by 120th min the glucose level did not return to the initial level. At the same time, administration of the polyphenolic extracts studied to healthy animals did not affect significantly this index.

However, the oral glucose load revealed some difference in the process of glucose utilization (Table). Thus, PE50Arg and PE50_cys administration caused a significant difference on the 90th min of OGTT in the BG level compared to IC group; it was caused by the probable stimulation of glucose uptake by cells.

Nevertheless, we were interested in studying the effect of the substances under research on glucose tolerance under IR experimentally induced. OGTT with AUC calculation is an informative test for assessing the potential hypoglycemic activity.

Fig. 1 presents the results of OGTT in rats with Dex induced IR and when administering polyphenolic extracts.
During OGTT in Dex group the glucose concentration maximized on the 60th min of the experiment, and by the 120th min remained significantly higher than the initial level, indicating the presence of IR. It was found that the AUC value in Dex group rats was at least 3 times more than the corresponding area in IC. It should be also noted that the oral administration of the substances studied for 14 days in Dex_PE50_arg and Dex_PE50_cys groups of rats led to a significant decrease in the BG concentration by 36.5 % and 35.1 %, respectively. Moreover, in the 90th min of the experiment the BG level in the groups mentioned significantly decreased, and in the 120th min it reached the initial level.

In HFD group (Fig. 2), the basal glucose level was significantly, in 1.69, times, higher than the values of the intact control. It was reflected in the AUC area that was twice more than of intact animals. PE50_arg and PE50_cys administration to IR animals normalized glucose levels. At the same time, the effect observed exceeded the effect of arph and was comparable with the action of met. OGTT in HFD_arg and HFD_cys groups showed that the BG elevation load, however, in rats that received PE50_arg and PE50_cys these indices were significantly lower compared to Dex group by 54.2 % and 51.8 %, respectively.

The maximum BG increase in the experimental groups was observed on the 60th min after glucose load, however, in rats that received PE50_arg and PE50_cys these indices were significantly lower compared to Dex group by 54.2 % and 51.8 %, respectively. Moreover, in the 90th min of the experiment the BG level in the groups mentioned significantly decreased, and in the 120th min it reached the initial level.
determined on the 30th and 60th min was significantly lower compared to HFD group, and by the 120th min decreased to the initial level. The effect of PE50_arg and PE50_cys observed by the 120th min after the glucose load was significantly higher than the same indicator of PF50 extract without amino acids. Therefore, OGTT showed the BG lowering on the 30th, 60th and 120th min of the test, indicating a significant decrease in the AUC area (Fig. 2).

Dexamethasone injections had a more pronounced effect on the glucose content compared to the HFD fructose diet, but it should be remembered that both models were effective. The general tendency observed when taking extracts from bearberry leaves studied showed that in 120 min they reduced BG to the initial level unlike the BG concentration that still kept higher in IR untreated animals.

These results can be the evidence of activation of glucose utilization processes, and it, in turn, indicates the insulin sensitivity improvement due to the action of the extracts studied. The effect observed is mediated by the presence of polyphenolic components that stimulate glucose uptake into cells, as well as the presence of amino acids that normalize signal transduction processes, which improve the insulin sensitivity of target cells.

CONCLUSIONS

1. The experimental study of antidiabetic properties of extracts from bearberry leaves has been performed under the experimental IR. The data obtained indicate that the corrective effect of arginine
and cysteine on signal transduction processes in insulin target cells plays an important role in the IR treatment.

2. It has been shown that PE50_arg and PE50_cys after two weeks of administration revealed the ability to decrease the blood glucose level in rats, as well as reduce IR development and improve tolerance to glucose under the experimental IR. The hypoglycemic activity has been found to be not much different from the action of Metformin, but exceeds the activity of Arphazetin.

3. Bearberry leaves are the promising raw material for creating an anti-diabetic drug. Thus, further study of the new polyphenolic extracts obtained on improving metabolic disorders is necessary.

Conflict of interests: authors have no conflict of interests to declare.

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Conflict of interests: authors have no conflict of interests to declare.

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Надійшла до редакції 30.03.2020 р.