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SCREENING OF BEARBERRY LEAVES EXTRACTS HYPOGLYCEMIC EFFECT AND STUDY OF ACUTE TOXICITY

Diabetes mellitus type 2 (DM2) is a global health problem. The plant origin medicinal preparations revealed different mechanisms of antidiabetic action. Despite a wide range of plants that have already been studied, other species are of interest. Particularly, Bearberry (*Arctostaphylos uva-ursi*) attracted our attention because its leaves are rich with biologically active compounds, but hypoglycemic activity has not been studied yet.

The aim of our experiment was studying acute toxicity and hypoglycemic activity of Bearberry extracts on non-diabetic standard models.

Materials and methods. It was conducted the screening of hypoglycemic effect in healthy rats, oral glucose tolerance test in healthy rats and study of acute toxicity. The object of study were water and alcohol polyphenol extracts of Bearberry leaves (extractor – alcohol 50 % and 90 %).

Results and discussion. Screening and comparative study shows that the maximal hypoglycemic activity revealed Bearberry leaves alcohol extract (extractor – 50 % ethanol, PE50) in dose 100 mg/kg. The least pronounced effect was observed for the introduction of water extract administration.

Conclusions. The results indicate the practicability of this Bearberry leaves extract further study for renewal the range of medicinal products that revealed hypoglycemic activity.

Key words: Bearberry (*Arctostaphylos uva-ursi*); acute toxicity; hypoglycemic activity; oral glucose tolerance test

Г. Б. Кравченко, Матар Мазен, О. А. Красильникова Скринінг гіпоглікемічної активності екстрактів з листя Мучниці звичайної та вивчення гострої токсичності

Цукровий діабет 2 типу (ЦД2) є глобальною проблемою охорони здоров'я. Ефективність лікарських препаратів рослинного походження пояснюється різними механізмами антидіабетичної дії. Незважаючи на широкий спектр рослин, які вже піддавалися вивченню, інтерес представляють і інші види. Зокрема, Мучниця звичайна (*Arctostaphylos uva-ursi*) привернула нашу увагу, так як її листя багате на біологічно активні сполуки, однак їх гіпоглікемічна активність ще не вивчена.

Мета даного експерименту полягала у вивченні гострої токсичності та гіпоглікемічної активності екстрактів Мучниці звичайної на стандартних моделях у здорових щурів.

Матеріали та методи. Було проведено дослідження гіпоглікемічної активності на здорових щурах, пероральний тест толерантності до глюкози на здорових щурах і дослідження гострої токсичності. Об'єктом дослідження були водний і спиртові поліфенольні екстракти листя мучниці звичайної (екстрагент – етанол 50 % і 90 %).

Результати та їх обговорення. Скринінг і порівняльне дослідження показують, що максимальну гіпоглікемічну активність проявив спиртовий екстракт з листя Мучниці звичайної (екстрагент – 50 % етанолу, PE50) в дозі 100 мг/кг. Найменший гіпоглікемічний ефект спостерігався при введенні водного екстракту.

Висновки. Результати показали доцільність подальшого вивчення екстракту листя Мучниці звичайної для оновлення асортименту лікарських препаратів з гіпоглікемічною активністю.

Ключові слова: Толокнянка звичайна (*Arctostaphylos uva-ursi*); гостра токсичність; гіпоглікемічна активність; пероральний тест толерантності до глюкози

А. Б. Кравченко, Матар Мазен, О. А. Красильникова Скрининг гипогликемической активности экстрактов из листьев Толокнянки обыкновенной и изучение острой токсичности

Сахарный диабет 2 типа (СД2) является глобальной проблемой здравоохранения. Эффективность лекарственных препаратов растительного происхождения объясняется различными механизмами антидиабетического действия. Несмотря на широкий спектр изученных растений, интерес представляют и другие виды. В частности, Толокнянка обыкновенная (*Arctostaphylos uva-ursi*) представляет научный интерес, так как ее листья богаты биологически активными соединениями, однако их гипогликемическая активность еще не изучена.

Целью нашего эксперимента было изучение острой токсичности и гипогликемической активности экстрактов Толокнянки обыкновенной на недиабетических стандартных моделях.

Материалы и методы. Было проведено исследование гипогликемической активности на здоровых крысах, пероральный тест толерантности к глюкозе на здоровых крысах и исследование острой токсичности. Объектом исследования были водный и спиртовые полифенольные экстракты листьев Толокнянки обыкновенной (экстрагент – алкоголь 50 % и 90 %).

Результаты и их обсуждение. Скрининг и сравнительное исследование показывают, что максимальную гипогликемическую активность проявил спиртовый экстракт из листьев Толокнянки обыкновенной (экстрагент – 50 % этанола, PE50) в дозе 100 мг/кг. Наименьший гипогликемический эффект наблюдался при введении водного экстракта.

Выводы. Результаты показали целесообразность дальнейшего изучения экстракта листьев Толокнянки обыкновенной для пополнения ассортимента лекарственных препаратов с гипогликемической активностью.

Ключевые слова: Толокнянка обыкновенная (*Arctostaphylos uva-ursi*); острая токсичность; гипогликемическая активность; пероральный тест толерантности к глюкозе

INTRODUCTION

Diabetes mellitus type 2 (DM2) is a global health problem. More than 230 million people worldwide are affected, and it is expected to reach 350 million by 2025 [1-2].

For the DM2 pharmacological correction clearly are used hypoglycemic drugs of synthetic origin but the naturally compounds complexes obtained from plants are quite popular. That is because their action involved different mechanisms and allows to prevent complications, which always occurs under the DM2 development [3-4]. Despite a wide range of plants that have already been studied, other species are of interest. In this respect our attention was attracted by Bearberry (*Arctostaphylos uva-ursi*), which leaves are rich with biologically active compounds and has been used for a long time in medical practice. However, this plant hypoglycemic activity was not studied [5]. For this purpose, we conducted Bearberry leaves extracts hypoglycemic screening in healthy (non-diabetic) rats and under glucose load (glucose tolerant test, GTT).

The aim of our experiment was studying acute toxicity and hypoglycemic activity of Bearberry extracts on non-diabetic standard models.

MATERIALS AND METHODS

Hypoglycemic study in healthy rats [6]. For screening of hypoglycemic activity were used inbred albino male rats (14 weeks age) weighing 180-200 g. Animals were divided into groups (n = 4) depending on the aim of the experiment: 1 – intact control (IC) healthy animals, which were administered physiological solution; 2-4 – animals administered water polyphenol extract of Bearberry leaves (PEW) in doses 50, 100, 500 mg/kg; 5-7 – animals administered 50 % Bearberry leaves ethanol extract (PE50) 50, 100, 500 mg/kg; 8-10 – animals administered 90 % Bearberry leaves ethanol extract (PE90) 50, 100, 500 mg/kg; 11 – animals administered Arphasetin infusion (AI) in recommended dose recalculated for rats (18 ml/kg). The test extracts were administered to overnight fasted animals orally with the help of gastric catheter sleeved to syringe. Blood glucose concentration were determined with the help of glucometer “One Touch Select” (LifeScan, USA) at the 0, 2, 4, 6 and 8 hours after extracts administration, samples were collected by gingival vein puncture [7].

Oral glucose tolerance test (OGTT) in healthy rats [6]. In order to study hypoglycemic activity using the OGTT were used inbred albino male rats (14 weeks age) weighing 180-200 g. Animals were divided into groups (n = 6) depending on the aim of the experiment: 1 – intact control (IC) healthy animals, which were administered physiological solution; 2 – animals, which were administered glucose solution in dose 3 g/kg body weight *per os* (glucose load, for other group of animals 30 min after drug administration); 3 – animals after glucose load and administered PEW; 4 – animals after glucose load and administered PE50; 5 – animals after glucose load and administered PE90; 6 – animals after glucose load adminis-

tered AI (18 ml/kg); 7 – animals after glucose load administered metformin (15 mg/kg). The test extracts were administered to overnight fasted animals orally with the help of gastric catheter sleeved to syringe in dose 100 mg/kg. Blood glucose concentration were determined with the help of glucometer “One Touch Select” (LifeScan, USA) at the 0, 15, 30, 60 and 120 minutes after glucose load, samples were collected by gingival vein puncture [7].

Study of acute toxicity. Acute toxicity of PE50 was determined according to the OECD (Organization for Economic Cooperation and development) No. 423 protocol (Protocol) [8]. In experiment were used female rats weighing 160-180 g, 14 weeks age (n = 3). According to the Protocol, study was done in two stages. During first stage, 3 groups of animals were administered 100, 600 and 1000 mg/kg of PE50 respectively intragastrically and observed for the first 4 h and 24 h for signs of toxicity and mortality. For the second stage were selected doses 2000, 3000 and 5000 mg/kg and 3 groups of rats after single ED50 intragastrically administration were supervised for signs of toxicity and mortality for 72 h.

All animals were kept in standard conditions of the NUPh Central Scientific-Research laboratory 7 days or more prior to allow for acclimatization. Animals were housed in polypropylene cages in groups of 3 animals room at 23 ± 2 °C and relative humidity of 50-60 % under 12 : 12 h light:dark cycle. The animals were provided standard diet and water ad libitum. The animal experiments were conducted after obtaining institutional ethics committee clearance, in accordance with the “General Ethical Principles of Animal Experiments” (Ukraine, 2001). Bearberry leaves extracts were obtained at NUPh pharmacognosy department under the prof. Koshoviy O. M. supervision.

The statistical processing of the data was carried out using the STATISTICA program (StatSoftInc., USA, version 6.0).

RESULTS AND DISCUSSION

The first stage of the experiment was done in order to screening and select the most effective extract and dose in non-diabetic rats for the assessment of hypoglycemic effect. We have found that more pronounced hypoglycemic effect was after 6 hours after treatment agents administration (Table). Nevertheless, it was indicated that studied extracts showed a hypoglycemic activity, its distinctiveness significantly depended on the extractor and dose used. Thus, hypoglycemic effect of PEW was not significant during all the time of observation. The significant decrease in blood glucose level was determined for PE50 in doses 50 mg/kg (8.1 % from the baseline), 100 mg/kg (14.4 % from baseline) and 500 mg/kg (7.2 % from baseline). As for PE90 introduction the maximal decrease in blood glucose content was observed also after 6 h from the extract administration, but only in the dose 100 mg/kg.

As a reference drug was selected Arphasetin, as, currently in the Ukraine pharmaceutical market, it is the only

Table

HYPOGLYCEMIC EFFECT OF BEARBERRY LEAVES EXTRACTS ON NORMOGLYCEMIC RATS

Group	Treatment	Dose (on kg of body weight)	Blood glucose, mmol/l				
			0	2 h	4 h	6 h	8 h
1	NaCl, 0.9 %	1 mg	3.97 ± 0.23	3.92 ± 0.22	4.04 ± 0.26	3.83 ± 0.18	3.85 ± 0.09
2	PEW	50	3.91 ± 0.18	3.96 ± 0.23	3.87 ± 0.22	3.91 ± 0.21	4.02 ± 0.14
3		100	4.04 ± 0.13	3.95 ± 0.19	4.01 ± 0.19	3.89 ± 0.19	3.87 ± 0.15
4		500	3.96 ± 0.19	3.89 ± 0.21	3.79 ± 0.14	3.87 ± 0.17	3.98 ± 0.23
5	PE50	50	4.05 ± 0.17	3.93 ± 0.17	3.81 ± 0.23	3.74 ± 0.24*	3.90 ± 0.21
6		100	4.01 ± 0.11	3.88 ± 0.24	3.61 ± 0.22*	3.43 ± 0.17*	3.72 ± 0.24
7		500	3.99 ± 0.24	3.94 ± 0.16	3.78 ± 0.19	3.70 ± 0.21*	3.81 ± 0.19
8	PE90	50	3.93 ± 0.15	3.84 ± 0.21	3.72 ± 0.24	3.65 ± 0.19	3.84 ± 0.18
9		100	3.89 ± 0.10	3.72 ± 0.18	3.67 ± 0.18	3.41 ± 0.14*	3.69 ± 0.21
10		500	4.06 ± 0.16	3.94 ± 0.22	3.71 ± 0.21	3.77 ± 0.09	3.93 ± 0.23
11	AI	18 ml	4.02 ± 0.22	3.63 ± 0.21	3.42 ± 0.24*	3.33 ± 0.21*	3.82 ± 0.14

Notes: * - $p < 0.05$ - Intact control vs treatment groups.

one plant origin preparation – plant species with anti-diabetic activity. In our experiment, we administered to laboratory animals the recommended dose recalculated for rats. The results showed the significant blood glucose rate reduction after 6 h after AI administration by 18.1 %. Thereby in normoglycemic study we have observed that glucose reduction rate was more prominent at a dose 100 mg/kg for PE50.

For further hypoglycemic activity study OGTT was carried out. Fig. depicts the result of OGTT in healthy rats after extracts oral administration.

As shown in Fig. the baseline levels of blood glucose did not differ among the groups. It was found that glucose load caused hyperglycemia by 60.3 % after 60 min. The administration of PEW did not have a significant effect

on the glycemia dynamics. On the contrary, PE50 administration caused a reliable glycemia after 30 minutes by 18.7 %, after 60 min by 26.8 % (Fig.). PE90 reduced glucose level in animals by 41.1 % in 60 min. Along with Arphasetin as reference preparation was used Metformin in dose 15 mg/kg.

As a result, determine the ability of Bearberry leaves extracts to reduce hyperglycemia after glucose load found that PE50 provides a significant reduction in glycaemia by 16.8. Thus, the most promising for further study should consider, therefore, further investigation of antidiabetic properties.

As for acute toxicity study, the obtained results showed that rats under administration of the PE50 study-doses the animal death and signs of intoxication were

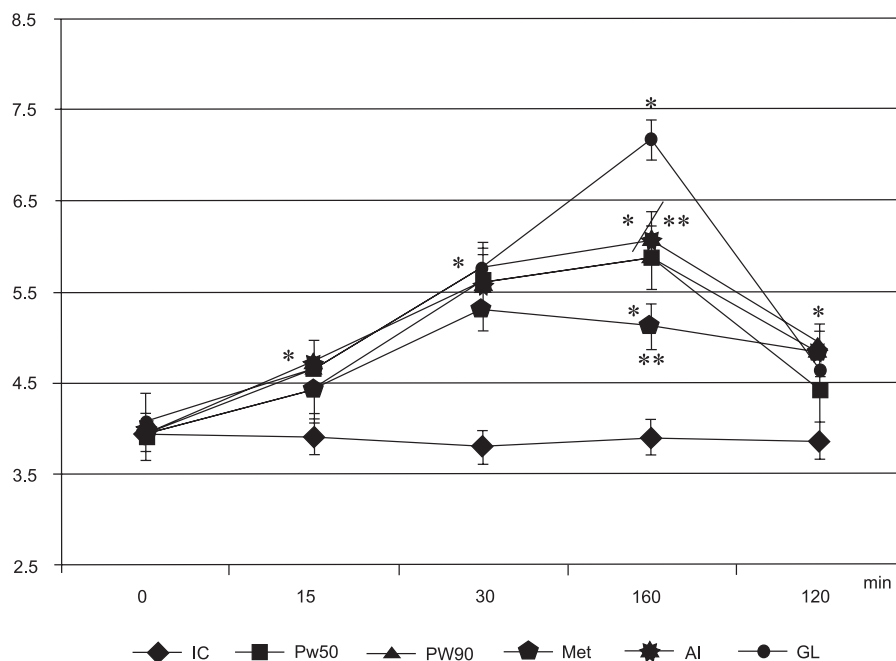


Fig. Effect of Bearberry leaves extracts on blood glucose level (mmol/l) in loaded normoglycemic rats

Notes: * - $p < 0.05$ - intact control vs treatment groups; ** - $p < 0.05$ - treatment groups vs glucose load

not observed. Thus, the substance belongs to the class of relatively non-toxic substances with intragastric administration according to the classification of toxicity [6].

CONCLUSIONS

Screening and comparative study shows that the maximal hypoglycemic activity revealed Bearberry leaves alcohol extract (extractor – 50% ethanol, PE50) in dose

100 mg/kg. PE50 belongs to the class of relatively non-toxic substances with intragastric administration.

The results indicate the practicability of this Bearberry leaves extract further study for renewal the range of medicinal products that revealed hypoglycemic activity.

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

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