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# INHIBITION OF PANCREATIC LIPASE BY WATER EXTRACTS OF SOME HERBAL MIXTURES

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**Abstract**

Diabetes mellitus is an important social and medical problem, as it causes the development of dangerous complications that lead to disability and mortality. This disease is characterized by a multi-vector pathogenesis that requires a comprehensive approach to treatment. Inhibition of pancreatic lipase activity is an important mechanism in the prevention and treatment of type 2 diabetes and obesity.

The aim of our research was to study an inhibitory α-glucosidase activity of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine and with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study *in vivo* and the defined phytochemical composition that determines such pharmacodynamics.

During the study of antidiabetic activity *in vitro* it was established the lipase IC50 was 756.46 μg/mL of the sample 1, 799.57 μg/mL of the sample 2, 812.71 μg/mL o of the sample 3, 769.56 μg/mL of the sample 4 and 712.21 μg/mL of the sample 5.

The present study showed a high inhibitory activity of herbal mixtures to pancreatic lipase, which suggests the effectiveness of the studied herbal mixtures for the prevention and treatment of type 2 diabetes and obesity.

***Keywords:*** diabetes mellitus, herbal mixtures, lipase activity, orlistat, obesity

## Introduction

Diabetes mellitus is a global social problem in the field of health care, due to rapid spread of this disease and the development of serious complications such as micro- and macroangiopathies, which significantly reduce the quality and life expectancy of patients [1]. According to the official information of International Diabetes Federation (2019), the number of patients is projected to increase to 642 million by 2040 [2].

One of the important problems of type 2 diabetes is such a complication as hyperlipidemia.

Pancreatic lipase is the most important enzyme responsible for digestion of dietary fat, slowing down the deposition of fat into adipose tissue and suppression of weight gain, which is of beneficial effects to overweight and obesity, which are common in diabetes [3, 4, 5, 6]. Pancreatic lipase is a key enzyme responsible for the digestion and absorption of triglycerides, which represent 90 to 95% of the ingested fats [7, 8, 9, 10]. If somehow this initial movement of triglycerides from the intestinal lumen is blocked, hyperlipidemia can be prevented [11, 12, 13, 14]. The application of a lipase inhibitor was examined earlier as a treatment for obesity. Orlistat, a hydrogenated derivative of lipstatin derived from *Streptomyces toxitricini*, a potent inhibitor of gastric, pancreatic and carboxyl ester lipase, has proved to be effective for the treatment of obesity, as a major factor in the development of type 2 diabetes [15, 16, 17, 18]. Management of hyperlipidemia without any side effects is still a challenge to the medical system. Due to risk of toxicity or adverse effect of drugs, the existence of lipase inhibitorsin various foodstuffs and natural products has been researched and the present of these inhibitors in cereals, wheat bran, wheat germ, soyabean and various herbs has been reported [19, 20, 21, 22].

It is for this reason that there is a need for natural lipase inhibitors that would possess no adverse or unwanted side effects [23, 24, 25, 26].

Particular attention deserves the combinations of different medicinal plants because such herbal mixtures will have more biologically active substances that will influence on all links of the pathogenetic mechanism of development of diabetes mellitus and its complications [27, 28, 29, 30].

Thus, the aim of our research was to study an inhibitory α- glucosidase activity of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 and with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study in vivo [23, 24, 25, 26] and the defined phytochemical composition that determines such pharmacodynamics [16, 17, 18, 19, 20, 21].

## Methods

**Plant materials:** The herbal raw materials harvested in June to August 2019 in Ternopil region (Ukraine) were used. After harvesting, the raw materials were dried, crushed and brought back to standard according to the general GACP requirements [31]. The plants were identified by Department of Pharmacognosy with Medical Botany, I.Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. The voucher specimens of the herbal raw materials have been deposited in Departmental Herbarium for future record.

For the study were used the five different herbal mixtures, composition of which is given in Table 1.

**Chemicals and standards:** chemical reference substance (CRS) of orlistat were of primary reference standard grade (≥ 95 % purity HPLC) and were purchased from Sigma-Aldrich Chemical Company (Germany), as well as lipase. Water used in the studies was produced by MilliQ Gradient water deionizaton system (USA).

**Extraction procedure:** the samples of herbal raw materials (10 g) were placed into a 100 mL conical flask with120 mL of distilled water. The extractions were carried out in a water bath for 30 min. The resulting extracts were filtered using Whatmann filter paper No1. Then the filtrates were evaporated by rotary evaporator and were lyophilized to dryness. The lyophilized powders of each herbal mixture were stored at 4 °C for further use.

**Inhibition of lipase enzyme:** the inhibitory activity against pancreatic lipase was measured using p-nitrophenyl butyrate as a substrate with a modified method. 10 µL of extracts (prepared at

concentrations of 100, 200, 400, 800 and 1000 µg/mL), positive control (Orlistat, 100 µM) and dimethyl sulfoxide (negative control vehicle used to dissolve the extracts) were pipetted into respective wells of a 96-well plate. Freshly prepared porcine pancreatic lipase was added at four times the volume of the test samples, positive and negative controls (40 µL). The plates were initially incubated at 37 °C for 15 minutes. Thereafter, 170 µL of the substrate solution was added to the wells. The plate was then incubated at 37 °C for 25 minutes.The absorbance was measured at 405 nm using the spectrophotometer Shimadzu 1800-UV (Japan). Experiment was performed in triplicate. [5].

**Calculation of 50% Inhibitory Concentration (IC50):** the inhibitory concentration of the water extracts of the herbal mixtures required to inhibit the activity of the enzyme by 50%, IC50 was calculated by regression analysis using the percentage scavenging activities at five different concentrations of the extracts. Inhibition (I %) was calculated by:

$$\%\, Inhibition = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## Results and Discussion

The primary role of lipase inhibitors is to decrease the gastrointestinal absorption of fats [32, 33, 34]. Fats then tend to be excreted in feces rather than being absorbed to be used as a source of caloric energy, and this can result in weight loss in individuals. These inhibitors could be used for the treatment of obesity, which can subsequently lead to type II diabetes and cardiovascular diseases if not managed [35, 36, 37]. Inhibitory effects against pancreatic lipase of water extracts of herbal mixtures by taking Orlistat as a positive control present in Table 2.

The relationship between the increase in the inhibitory activity of pancreatic lipase and the concentration of aqueous extracts of herbal mixtures was revealed. It was experimentally established that the concentration required for 50% inhibition (IC50) of lipase enzyme was 756.46 µg/mL of the sample 1; 799.57 µg/mL of sample 2; 812.71 µg/mL of the sample 3; 769.56 µg/mL of the sample 4; 712.21 µg/mL of the sample 5 (Table 2). The IC50 value of standard drug Orlistat against lipase was 94.12 µg/mL.

Inhibition of intestinal pancreatic lipase are used for tretment of diabetes mellitus type 2 to reduce hyperlipidemia [38]. The search for a new pancreatic lipase inhibitor from herbal mixtures is a striking method for the management of obesity. Primely metabolites such as polyunsaturated fatty acids that possess lipase inhibitory activity [39].

## Conclusions

For the first time, it was conducted the study of pancreatic lipase inhibition in the water extracts of the herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 and with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study *in vivo* and the defined phytochemical composition that determines such pharmacodynamics. The present study showed a high inhibitory activity of herbal mixtures to pancreatic lipase, which is one of the mechanisms of prevention and treatment of type 2 diabetes and obesity.

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**Table 1. Composition of the herbal mixtures**

| Herbal mixtures | Herbal drug component | Portion in the mixture, % | Relative ratio |
|---|---|---|---|
| Sample 1 | *Urtica dioica* leaf | 26.32 | 5 |
| | *Cichorium intybus* roots | 26.32 | 5 |
| | *Rosa majalis* fruits | 21.05 | 4 |
| | *Elymus repens* rhizome | 15.79 | 3 |
| | *Taraxacum officinale* roots | 10.52 | 2 |
| Sample 2 | *Arctium lappa* roots | 26.32 | 5 |
| | *Elymus repens* rhizome | 26.32 | 5 |
| | *Zea mays* columns with stigmas | 21.05 | 4 |
| | *Helichrysum arenarium* flowers | 15.79 | 3 |
| | *Rosa majalis* fruits | 10.52 | 2 |
| Sample 3 | *Inula helenium* rhizome with roots | 10.0 | 1 |
| | *Helichrysi arenarium* flowers | 20.0 | 2 |
| | *Zea mays* columns with stigmas | 20.0 | 2 |
| | *Origanum vulgari* herb | 20.0 | 2 |
| | *Rosa majalis* fruits | 20.0 | 2 |
| | *Taraxacum officinale* roots | 10.0 | 1 |
| Sample 4 | *Cichorium intybus* roots | 26.32 | 5 |
| | *Elymus repens* rhizome | 26.32 | 5 |
| | *Helichrysum arenarium* flowers | 21.05 | 4 |
| | *Rosa majalis* fruits | 15.79 | 3 |
| | *Zea mays* columns with stigmas | 10.52 | 2 |
| Sample 5 | *Urtica dioica* leaf | 20.0 | 1 |
| | *Taraxacum officinale* roots | 20.0 | 1 |
| | *Vaccinium myrtillus* leaf | 20.0 | 1 |
| | *Rosa majalis* fruits | 20.0 | 1 |
| | *Mentha piperita* herb | 20.0 | 1 |

**Table 2. Lipase inhibition of water extracts of the samples of the herbal mixtures**

| Herbal mixtures | Concetration, µg/mL | Inhibition, % | IC50, µg/mL |
|---|---|---|---|
| Sample 1 | 100 | 30.69± 3.16 | 756.46 |
| | 200 | 31.16± 3.13 | |
| | 400 | 44.95± 2.68 | |
| | 800 | 52.10± 3.68 | |
| | 1000 | 63.86± 3.08 | |
| Sample 2 | 100 | 29.49± 2.93 | 799.57 |
| | 200 | 39.58± 3.14 | |
| | 400 | 42.64± 3.17 | |
| | 800 | 50.86± 3.23 | |
| | 1000 | 61.46± 3.88 | |
| Sample 3 | 100 | 28.94± 3.07 | 812.71 |
| | 200 | 30.48± 2.64 | |
| | 400 | 43.82± 2.74 | |
| | 800 | 50.69± 3.02 | |
| | 1000 | 60.93± 3.17 | |
| Sample 4 | 100 | 29.83± 3.04 | 769.56 |
| | 200 | 38.94± 3.12 | |
| | 400 | 41.99± 3.05 | |
| | 800 | 54.04± 2.61 | |
| | 1000 | 60.96± 2.16 | |
| Sample 5 | 100 | 20.85± 3.08 | 712.21 |
| | 200 | 33.91± 3.23 | |
| | 400 | 47.07± 3.30 | |
| | 800 | 55.19± 2.93 | |
| | 1000 | 65.38± 2.85 | |
| Orlistat (standart) | 100 | 42.19± 2.13 | 94.12 |
| | 200 | 59.84± 1.94 | |
| | 400 | 72.11± 1.594 | |
| | 800 | 84.09± 2.32 | |
| | 1000 | 89.73± 2.63 | |

Note: Values are expressed as mean ± SD (n=3).