Hypoglycaemic and cholesterol lowering properties of an extract obtained from *Vaccinium myrtillus* leaves and *Humulus lupulus* cones

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**Abstract**

Glycaemia represents the glucose level in the blood. Pathologically increase of the level of glycaemia occurs when the pancreas does not secrete enough insulin which may lead to diabetes and obesity. Long-term hyperglycaemia also leads to vascular and neurological complications. In developed countries, interest in the use of herbal products has increased recently due to the fact that the scientific evidence regarding their effectiveness has become widely available. The aim of this paper is to study the hypoglycaemic and cholesterol lowering properties of an combined extract obtained from a *Vaccinium myrtillus* leaves and *Humulus lupulus* cones 1:1 (mass) mixture. To the best of our knowledge, this combination has not been tested experimentally in this regard. Our results show that the administration of 1 ml (5 g extract - 100ml) day/ 100g body weight / day to rats with diabetes induced by 60mg/Kg body of STZ, for 4 weeks, leads to a decrease in preprandial glucose by 21% and in total serum cholesterol by 7%.

**Keywords:** *Vaccinium myrtillus, Humulus lupulus*, hypoglycaemic, cholesterol lowering

1. Introduction

Glycaemia represents the glucose level in the blood (1). Feedback mechanism of the body maintains blood glucose levels relatively constant, at values of around 1 g of glucose per liter. Required amount of blood glucose necessary for organs and tissues are insured by blood circulation.

Hormonal substances regulate blood sugar level. Insulin is the substance that decreases blood glucose and glucagon, growth hormone and adrenaline increases blood glucose levels. Increased glucose above the normal values (à jeun - on an empty stomach) at levels exceeding 140 mg / dl is hyperglycaemia. Postprandial hyperglycaemia (hyperglycaemia for a short time) can occur after meals rich in carbohydrates, poisoning, stress, and fever. Pathologically increase of the level of glycaemia occurs when the pancreas does not make enough insulin which may lead to diabetes and obesity. Long-term hyperglycaemia also leads to vascular and neurological complications. According to the World Health Organization, currently in the world 280 million people are diagnosed with diabetes, and of these, 55.2% live in Europe. According to forecasts, in the next 20 years this number will increase to 380 million. Doctors draw attention to the increasing frequency of diabetes among children and adolescents by 3% each year, and at 5% per year in preschool children. It is also increasing the frequency of type II diabetes in adolescents and youth.

The use of natural products is very common among non-industrialized societies because these remedies are more affordable at lower prices compared to modern pharmaceuticals. In developed countries, interest in the use of herbal products has increased recently due to the
fact that the scientific evidence regarding their effectiveness has become widely available (2).

Herbal products have been known to mankind since ancient times (3). Plants were the primary source of drug development, and many currently available were directly or indirectly derived from plants. For example one of the substances used in hypoglycaemic antidiabetic medications - metformin, is derived from *Galega officinalis* (4).

Currently there are about 800 species of plants with hypoglycaemic properties (anti-diabetic) (5).

**Blueberry**- *Vaccinium myrtillus* L., fam. *Ericaceae*, is a native shrub. In the leaves of bilberry have been identified, 35 polyphenolic compounds including flavan-3-ols, proanthocyanidins, flavonols and their glucosides and also conjugated phenolic acids (6). A comparative study on phenolic composition of harvested and dried leaves of blueberries to those purchased commercially, revealed a few differences in the concentration respectively: for spectrophotometrical determination - total phenolic compounds 12.98 and 10.62%, tannins 7.84 and 7.43%, total flavonoid compounds 2.98 and 2.20% and for HPLC determination the differences were respectively: total flavonoid compounds 1.41 and 1.16%, quercetin 3-glucuronide 1.02 and 0.83%, hyperoside 0.22 and 0.16%, chlorogenic acid 3.66 and 1.58% (7). Also, the content in flavonoids determined by HPTLC with densitometry detection for an extract of blueberry leaves was measured to 134mg of isoquercetin and 450mg of hyperoside per gram of dried leaves (8).

Even if blueberry is most often promoted for its potential to improve vision, studies have demonstrated efficacy of this species in lowering blood glucose, also having anti-inflammatory, lipid-lowering and antioxidant properties by reducing oxidative stress. Therefore, blueberries has a great potential value in the treatment or preventing conditions associated with inflammation, dyslipidaemia, hyperglycaemia or increased oxidative stress, cardiovascular disease, cancer, diabetes, dementia and other diseases of the 3rd age (9). *Vaccinium* species are assigned with hypoglycaemic properties and are used in traditional medicine to relieve the symptoms of diabetes. Most studies conducted to date on the leaves of bilberry focuses on the hypoglycaemic effect of polyphenolic compounds, especially anthocyanins. (10).

**Hops** - *Humulus lupulus* L., is part of the fam. *Canabaceae*. Major constituents of hop are bitter substances (15-25%) found in resins. Chemical composition of hops includes volatile oils, resins, tannins, flavones (rutin, quercetin), chalcone (xanthohumol), flavanones (izoxantohumol), choline, asparagine, trimethylamine, p-aminobenzoic acid and 8% mineral substances (11). Hop plant is known for its use as a raw material in the brewing industry. Hop cones, rich in polyphenolic compounds and acyl-floroglucide are used extensively for conservation and to give beer its aroma and characteristics flavor. The hop cones have been used for a long time in medical purposes. In particular, it was recommended for the treatment of sleep disorders (12).

Up to now there are few studies on the hypoglycaemic effect of hops. Isohumulones isolated from hops have demonstrated this effect in both animal and in humans studies (13, 14)

This article investigates the hypoglycaemic and cholesterol lowering properties of an combined extract obtained from a 1:1 (mass) mixture of species *Humulus lupulus* and *Vaccinium myrtillus*. Considering both traditionally use of bilberry leaf and studies for the two species regarding the hypoglycemic potential as well as the content of total polyphenolic compounds and significant antioxidant effect of this combined extract (15), the results obtained in this paper can be a first step toward obtaining and possibly entering in the specialized market of a new bioproduct. Must be considered, that bilberry leaves are used, leading to a higher recovery of secondary horticultural products and also at enlargement of the area use of hops in therapeutic purposes.
Hypoglycaemic and cholesterol lowering properties of an extract obtained from *Vaccinium myrtillus* leaves and *Humulus lupulus* cones

2. Materials and methods

**Raw materials:** *Humulus lupulus* L. (cones), *Vaccinium myrtillus* L. (leaves) - were purchased from Plafar (Romania)

**Preparation of plant extracts** –

The combined extract (P) was obtained in the same manner as (15), by: extraction with ethyl alcohol 50%: vegetal material (*Vaccinium myrtillus* leaves:*Humulus lupulus* cones 1:1 (mass)/ solvent rate 1:15 (w/v) at boiling temperature for 3 hours, under continuous stirring. After filtration the solution was concentrated under reduced pressure at 60°C to a vegetal material/water extract rate - 1:1 (w/v) and spray dried.

**HPTLC Analysis for Phloroglucinol derivates**

The technique used for identification of phloroglucinol derivates have been performed according and comparative to TLC Atlas - *Plant Drug Analyses* (16). The development of characteristic finger printing profile was performed by densitometry HPTLC analysis. The extracts were dissolved with HPLC grade 50% (v/v) methanol to 1% (w/v) solution concentration. Then, 2-3µl of the sample was loaded as 10mm band length in the 20 x 10 Silica gel 60F254 TLC plate. The mobile phase was constituted of -acetic acid -ethyl acetate -cyclohexane 2:38:60 (v/v/v/v). After development, plates were air dried. The fingerprints were evaluated at 366nm in fluorescence mode with a WinCats and VideoScan software.

**Experimental animals**

The animals (Wistar male rats weighing 200 ±10g) used for the studies were divided into three groups (n=7).

The use and maintenance of laboratory animals was made in compliance with the provisions and regulations in the field issued by FELASA (Federation of European Laboratory Animal Science Associations) and taken over by Arsal (Romanian Association for Laboratory Animal Science) currently in force.

Prior to the experiments, the animals were fed with standard laboratory food for one week in order to adapt to the laboratory conditions. 12 hours before the test the animals were kept in fasting being allowed only access to water.

**Hypoglycaemic effect test**

There were three equal groups (n = 7), homogeneous for each test sample, namely:

Lot 1 - LC control group ;
Lot 2 - LD group - group with diabetes induced by streptozotocin 60mg/Kg body
Lot 3 - LDP group - group with diabetes induced by streptozotocin 60mg/Kg body, treated with test sample P -1 ml homogenate / 100g body weight/day

**Administration of substances**

The experiment was conducted over a period of 4 weeks.

Induction of diabetes was made by administration of streptozotocin (STZ) (98% (HPLC) Sigma Aldrich) at a single dose of 60mg/Kg body, 10 mM Na citrate solution (pH 4.5) intraperitoneally. In order to prevent hypoglycaemic shock to streptozotocin-induced diabetic animals 20% glucose solution in the drinking water during the first 24 hours was administrated (17).

Administration of plant product was achieved by intragastric gavage of 1 ml homogenate = 5 g - 100ml distilled water) / 100g body weight/day.

At 24 hours after the last experimental treatments and after starvation for 18 hours, drinking water was allowed ad libitum, animals were sacrificed under the terms of the current rules (18). The preprandial glucose and total serum cholesterol levels were determined. Evolution of the weight of the animals of each group was followed throughout the experiment, weight of starvation was recorded at sacrifice.

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Determination of glucose level: 100 μl of collected blood was haemolysed in 1 ml double distilled water. Deproteinization of the samples was carried out by adding 0.2 ml of 0.3 N barium hydroxide and 0.2 ml of 5% zinc sulphate according to the classical method of Nelson (1944) (19). After centrifugation for 20 minutes at 3500 rpm, glucose was determined in 0.25 ml of supernatant by the method of GOD-Perid (20), by using Test-Combination Glucose Kit (Boehringer, Gmbh, Mannheim, Germany). Optical density readings of the samples were taken at a Spekol 210 (Analytic Jena) UV-VIS spectrophotometer at 610 nm, blood glucose was expressed as mg of glucose / dL whole blood.

Determination of total serum cholesterol:
Total serum cholesterol was determined from 50 microliters of blood serum (21), using 3 ml of glacial acetic acid and 2 ml of iron trichloride in concentrated sulphuric acid. Pink-violet coloration of the samples and standard cholesterol sample was read at a Spekol 210 (Analytic Jena) UV-VIS spectrophotometer at 560 nm, using as reference two samples each consist in 50 μl of cholesterol standard with a concentration of 100 mg / 100 ml.
Samples values are expressed in mg of total serum cholesterol / dL serum.

Statistical Analysis.
The results were expressed as means ± S.D and the difference was tested by Student’s t-test. P-values lower than 0.05 were considered statistically significant.

3. Results and dissuctions

*HPTLC Analysis for Phloroglucine derivates*
According to HPTLC profile obtained for the combined extract P, comparative with Plant Drug Analysis chromatogram the presents of humulone (Rf~0.45) and lupunone (Rf~0.26) are reveald in figure 1.

*Figure 1.* HPTLC - Phloroglucine derivates chromatogram of: A- P; B- *Humulus lupulus* extract - Plant Drug Analysis (16)
Hypoglycaemic and cholesterol lowering properties of an extract obtained from *Vaccinium myrtillus* leaves and *Humulus lupulus* cones

**Hypoglycaemic effect test**

Over the years animal models have been developed for the study of diabetes or for testing anti-diabetic compounds. Those models of induction of diabetes in certain species of animals include chemical compounds, surgery (pancreatectomy) and genetic manipulation. Diabetogenic drugs include alloxan monohydrate, streptozotocin with or without nicotinamide, ferric nitrilo-triacetate, dithizone and anti-insulin serum. Chemical induction of diabetes is carried out mainly by two specific drugs namely streptozotocin (STZ - 69%) and alloxan (31%).

In the experiment, for male Wistar rats, preprandial blood glucose level after 18 hours of fasting had average values of 73.57 mg / dl. For the group to which diabetes was induced with STZ, mean preprandial glucose level were 110.86 mg / dl. The increase in preprandial glucose level between the two groups was of approximately 33%.

The LDP group to which diabetes was induced on the same conditions as for the LD group, but was treated with the test sample P, 1 ml of homogenate / 100g body weight / day respectively 50 mg extract / 100 g body weight / day, mean blood glucose level was 87.57 mg /dl (Table 1, Figure 2). The decrease in mean preprandial blood glucose levels between the two groups LD and LDP being of 21%. Decrease in preprandial glucose level at a rate of 21% for LDP compared to LD is significant, the mean values approaching normal blood glucose levels recorded for control group.

<table>
<thead>
<tr>
<th>No.</th>
<th>LOT</th>
<th>LC</th>
<th>LD</th>
<th>LDP</th>
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<td>Blood glucose mg/dl</td>
<td>73.57</td>
<td>110.86</td>
<td>87,57</td>
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<td>Mean value</td>
<td>Standard deviation</td>
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<td>Confidence interval 95%</td>
<td>68.62 to 78.52</td>
<td>102.97 to 118.75</td>
<td>83,34 to 91,80</td>
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</table>

The mean value of total serum cholesterol in the control group - for untreated animals, was 275.14 mg / dl and that in STZ-induced diabetic group was 320.57 mg / dl, leading to a
growth of 14.21% (Table 2, Figure 3). Administration of the extract P resulted in a reduction in mean total serum cholesterol by 7% to 295.71 mg / dl, compared to LD group. Weight variation of the animals had no significant values.

<table>
<thead>
<tr>
<th>No.</th>
<th>LOT</th>
<th>LC</th>
<th>LD</th>
<th>LDP</th>
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<tr>
<td></td>
<td></td>
<td>Total serum cholesterol mg/dl</td>
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<td></td>
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<tr>
<td>1</td>
<td>278</td>
<td>350</td>
<td>300</td>
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<td>2</td>
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<tr>
<td>7</td>
<td>287</td>
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</tr>
<tr>
<td>Mean value</td>
<td>275.14</td>
<td>320.57</td>
<td>295.71</td>
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</tr>
<tr>
<td>Standard deviation</td>
<td>12.62</td>
<td>31.92</td>
<td>13.63</td>
<td></td>
</tr>
<tr>
<td>Confidence interval 95%</td>
<td>263.48 to 286.81</td>
<td>291.05 to 350.09</td>
<td>283.10 to 308.32</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Total serum cholesterol values obtained for the three experimental groups

**Figure 2.** Preprandial glycaemia values for the three experimental groups

**Figure 3.** Total serum cholesterol values for the three experimental groups

4. Discussions

The aim of this paper was to study the hypoglycaemic and cholesterol lowering activities of an combined extract obtained from a *Vaccinium myrtillus* leaves and *Humulus lupulus* cones 1:1 (mass) mixture. To the best of our knowledge this combination has not been tested...
experimentally in this regard. Our results show that the administration of 1 ml of homogenate / 100g body weight / day respectively 50 mg extract / 100 g animal / day, to rats with diabetes induced by STZ to 60mg/Kg body, for 4 weeks, leads to a decrease in preprandial glucose by 21% and in total serum cholesterol by 7%.

Similar results were obtained after oral administration of a dried hydroalcoholic extract of blueberry leaves, in rats with streptozotocin-induced diabetes, for four days, the serum glucose level (as determined during any time of the day at 2 hours after induced hyperglycaemia with the intake of glucose dissolved in water) decreased significantly by 26% in two different stages of diabetes (22). It must be taken into account the fact that the induction of diabetes with STZ takes a while and experiment conducted at appropriate time intervals after administration of the agent will provide information on the mechanism of action of the compound tested. In the first part of the induction, hypoglycaemic activity of plant extracts may be due to stimulation of residual activity of $\beta$ cells; after induction of diabetes status, hypoglycaemic activity is due to a different mechanism because in this model of diabetes insulin activity is neglected (23).

Also in a double-blind, placebo-controlled study of 94 human subjects with prediabetes according to the diagnostic criteria of the Japanese Society of diabetes, that were given capsules containing isohumulones has caused a decrease in fasting (preprandial) blood glucose level after 4 weeks of treatment and body mass index decreased significantly after 12 weeks of treatment (24).

The cholesterol lowering effect of this extract is not be neglected as it is possible that on an administration for a period longer than 4 weeks, the cholesterol-lowering effect to be more pronounced.

The antioxidant properties of the combined extract investigated by us in the previous article is also important in the context of the fact that it is now accepted that hyperglycemia-mild, chronic or fluctuating is a pro-oxidative condition (25).

5. Conclusions

Given the results obtained it can be concluded that the active principles from the blueberry leaves and hop cones have hypoglycaemic and cholesterol-lowering properties and this extract can be used as an adjuvant, respectively natural alternative, in hypoglycaemic and cholesterol-lowering therapies.

References

1. REPORT OF A WHO/IDF CONSULTATION 2006 - Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia - -Printed by the WHO Document Production Services, Geneva, Switzerland
2. SALIMIFAR, MOHSEN; FATEHI-HASSANABAD, ZAHRA; FATEHI, MOHAMMAD A Review on Natural Products for Controlling Type 2 Diabetes with an Emphasis on their Mechanisms of Actions Current Diabetes Reviews Volume 9, Number 5, September 2013 , pp. 402-411(10) Bentham Science Publishers


