The aim of this study was to investigate influence of the preparation based on barley and brewer’s yeast extracts with chromium (BBCr) and stevioside (S) on fasting glycaemia and glycaemia in mice after glucose, adrenalin and alloxan application. The animals were divided into three groups: glucose 500 mg kg⁻¹ (I); adrenalin 0.2 mg kg⁻¹ (II) and alloxan 100 mg kg⁻¹ (III) and into subgroups according to the substance they received: stevioside 20 mg kg⁻¹ (I-S, II-S, III-S); BBCr 750 mg kg⁻¹ (I-BBCr, II-BBCr, III-BBCr) and saline 1 ml/100g (III-placebo). Glycaemia was measured before and after 7-day treatment with stevioside or BBCr in the following conditions: fasting, 30 min after glucose load (I) or 45 min after adrenalin load (II). In group III glycaemia was measured before and after 12-day treatment with S, BBCr or placebo and alloxan application (7th, 8th and 10th days of treatment). BBCr significantly reduced fasting glycaemia in I and II groups and glycaemia values after the glucose load (I-BBCr: 1.53 ± 0.61 vs. 1.75 ± 0.83 mmol/L; p = 0.01). Stevioside significantly reduced glycaemia after the adrenalin load (II-S: 13.45 ± 0.71 vs. 11.65 ± 1.19 mmol/L; p = 0.03). In the III-BBCr glycaemia values did not indicate the development of alloxan-induced diabetes and were significantly lower than in the III-placebo (8.6 ± 3.16 vs. 18.8 ± 5.53 mmol/L; p < 0.05). In conclusion, BBCr caused a significant decrease of fasting glycaemia, significant reduction of glycaemia after glucose load and prevented onset of alloxan-induced diabetes. Stevioside caused the decrease of adrenalin-induced hyperglycaemia.

KEY WORDS: stevioside, barley and brewer’s yeast extract, alloxan, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a serious global health problem that significantly affects quality of life and lifespan. Plants which are traditionally used in diabetes could become a source of new oral anti-hyperglycaemic compounds, or act as an adjunct therapy. The World Health Organization Expert Committee on Diabetes has suggested an investigation of the traditional methods for the treatment of diabetes [1]. Consequently, the discovery of antidiabetic factors contained in natural substances has been the subject of intense research. More than 400 kinds of plants are reported to play a role in reducing the blood sugar levels [2]. The plant Stevia rebaudiana (Bertoni) Bertoni is used in the traditional treatment of diabetes among the Guarani Indians in Brazil and Paraguay. The diterpene glycoside, stevioside isolated from Stevia rebaudiana (Bertoni), exhibits a direct insulinotropic action in both isolated mouse islets and the clonal beta-cell lines (INS-1) [3,4] and possesses insulinotropic, glucagonostatic and anti-hyperglycemic effects in diabetic animals [5,6]. In experimental animals and clinical studies barley products have shown preventive and therapeutic antidiabetic properties. In addition to conventionally malted barley, barley can be fermented using barley mixed with brewer’s yeast. The research into physiological activities of this material is just in the beginning stage [7]. The preparation based on barley and brewer’s yeast extracts enriched with organically bound chromium (BBCr) is an auxiliary medicine registered in the Republic of Serbia in 2005 as a diabetic supplement in the treatment of insulin-independent diabetes mellitus. The supposed mechanism of its action consists of the activation of adenosine 5-monophosphate kinase (AMPK) which, via a series of chemical reactions, leads to an increased expression of the GLUT-4 receptor on the membranes of muscle cells and increased take-up of glucose [8]. Organically bound chromium normalizes the permeability of cell membranes to glucose, regulates biochemical processes of its utilization and deposition in the cells, and thus acts synergistically with insulin [9]. This study is concerned with the hypoglycaemic action of stevioside and BBCr in mice with glucose, adrenalin, or
alloxan induced hyperglycaemia. Alloxan (2,4,5,6-tetraoxypryrimidine; 5,6-dioxypyrazin) is widely used to induce experimental, insulin dependent, diabetes in animals. The toxic action of alloxan on pancreatic beta cells is the sum of several processes such as oxidation of essential -SH groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis [10]. There is limited number of published studies concerning the investigation of the efficacy of a combination of barley and brewer’s yeast extracts enriched with chromium in reduction of blood glucose levels in laboratory animals with experimentally induced hyperglycaemia. In this point of view, the aim of this study was to investigate and compare influence of auxiliary medicine BBCr and stevioside on fasting glycaemia and to investigate their efficacy in normalisation of glycaemia in mice with hyperglycaemia induced in three different ways (after glucose load, adrenalin load and alloxan application).

**MATERIALS AND METHODS**

**Experimental animals**  
Experiments were carried out on NMRI-Haan laboratory mice of both sexes, body weight 21.0 - 46.2 g, more than 3 months old. The animals had free access to food and water and a 12 hour succession of light and dark periods at 22 °C. The local University Ethic Committee at the Faculty of Medicine in Novi Sad approved all animal protocols. Laboratory animals were in human care in accordance with the criteria of the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institutes of Health [11].

**Chemicals**  
Glucose-anhydrous: a glucose dose of 500 mg kg⁻¹ was used in the oral glucose load test. According to the long-standing experience of our laboratory, this glucose dose proved to be adequate in mice in testing the potential hypoglycaemic action of medicinal herbs, because it indicates the effectiveness of the tested drug in this regard [12,13]. Adrenalin-hydrochloride: a dose of 0.2 mg kg⁻¹ s.c. was used for adrenalin induced hyperglycaemia. Alloxan: alloxan was administered intraperitoneally in 3 doses of 100 mg kg⁻¹ each, which were administered in the interval of 24 h (between 1st and 2nd dose), i.e. 48 h (between 2nd and 3rd dose) [14].

**Auxiliary medicines**  
Stevioside: prepared stevioside solution was administered by the oral probe in the dose of 20 mg kg⁻¹ (1 ml/100 g), i.e. 20 mg was dissolved in 10 ml of saline solution (0.2% solution). BBCr: the active ingredient of this product is a fermentation product of barley and brewer’s yeast (Glut 4 plus - insulin sensitizer) with the percentage of tablet composition of 76.92%. The product is enriched with organically bound chromium in concentration of 28.1 ± 5 mg/100 g. Excipients are: manitol, starch, lactose, cellulose, microcrystalline, sodium stearyl fumarate, acesulfame, chocolate and coffee flavour. Prepared solution of BBCr was administered by oral probe in the dose of 750 mg kg⁻¹ (1 ml/100 g), i.e. 1000 mg was dissolved in 13.3 ml of saline solution. The doses of stevioside and BBCr were calculated according to the human dosage.

**Apparatus**  
Accu-Check instrument (F.Hoffmann - La Roche Ltd., Basel, Switzerland): glycaemia was measured by means of test strips, which contained specific glucose oxidase enzyme for detection of glucose present in the blood. Concentration was expressed in mmol/L.

**Experimental procedure**  
The animals were randomized into three main groups, according to hyperglycaemia induction (I group: glucose, II group: adrenalin or III group: alloxan). The groups were divided into subgroups with respect to the substance they received (stevioside or BBCr): subgroups I-S, I-BBCr, II-S, II-BBCr, III-S, III-BBCr and III-placebo (saline, 1 ml/100g p.o). Each subgroup consisted of 6 animals and each animal was its own control in respect of glycaemia before and after the treatment with test substances, except for group III (alloxan), which contained a placebo subgroup. Glycaemia in mice was measured in the drop of capillary blood, obtained by incision of the tail tissue near its top. Blood drop was placed on the indicator strip of the Accu-Check instrument and glycaemia value in mmol/L was read. While capillary blood from the tail was taken, mice were held carefully, wrapped in a towel, and care was to minimise distress. Before beginning of the experiment the animals body mass and fasting glycaemia was measured and recorded in all three groups (glycaemia before treatment). After that, the animals of Group I were loaded with glucose (500 mg kg⁻¹ p.o.) and glycaemia was measured 30 min later (glucose load glycaemia before treatment). During the subsequent 7 days subgroups I-S and I-BBCr received stevioside or BBCr, respectively. After 7-days of treatment, the animals of subgroups I-S and I-BBCr were tested again for fasting glycaemia and glycaemia after glucose load. The same experimental procedure was applied to the animals of Group II with the difference that these animals were loaded with adrenalin (0.2 mg kg⁻¹ s.c.) and glycaemia was measured 45 min later. Measuring time of glycaemia after glucose and adrenalin load (30 and 45 min) was selected based on the data of the research conducted in the Department of Pharmacology, Toxicology and Clinical Pharmacology of the Faculty of Medicine in Novi Sad [15].
After measurement of fasting glycaemia, the animals of Group III were treated with stevioside (subgroup III-S), BBCr (subgroup III-BBCr) and placebo (subgroup III-placebo) during 15 days. The placebo subgroup was given perorally with 1 ml/100 g saline. After 7-days of treatment the animals of Group III received the first dose of alloxan (100 mg kg⁻¹) and glycaemia was measured 24 h after that. These animals received another two doses (on the 8th and 10th day) of alloxan, and glycaemia was measured 48 h later (Figure 1) [14].

**Statistical analysis**

Statistical analysis of data was carried out using paired samples t-test with the error level < 0.05 and ANOVA test. All data are reported as the X ± SD.

**RESULTS**

As presented in the table 1, in group loaded with glucose (group I), stevioside (subgroup I-S) significantly changed the fasting glycaemia levels after the 7-day treatment (7.38 ± 0.2 mmol/L vs. 6.27 ± 0.15 mmol/L; p = 0.03). Stevioside did not reduce glycaemia in mice after the glucose load comparing with the values before treatment. Seven-day treatment with BBCr (subgroup I-BBCr) resulted in significant reduction of fasting glycaemia levels (7.05 ± 0.2 mmol/L vs. 6.85 ± 0.99 mmol/L; p = 0.01).

**TABLE 1.** The effects of stevioside and preparation based on barley and brewer’s yeast extracts enriched with chromium (BBCr) on hyperglycaemic response to glucose load (500 mg kg⁻¹, p.o.) in mice

<table>
<thead>
<tr>
<th>Group I</th>
<th>Subgroup I-S (n = 6) - Stevioside 20 mg kg⁻¹ p.o. (7 days)</th>
<th>Subgroup I-BBCr (n = 6) - BBCr 750 mg kg⁻¹ p.o. (7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glycaemia (glycaemia before treatment)</td>
<td>Glucose load (500 mg kg⁻¹, p.o.) and measurement of glycaemia 30 min later (glucose load glycaemia before treatment)</td>
<td>Fasting glycaemia (glycaemia after treatment)</td>
</tr>
</tbody>
</table>

Results are presented as X±SD. Subgroup I-S: 7-day treatment with stevioside (20 mg kg⁻¹, p.o.); Subgroup I-BBCr: 7-day treatment with BBCr (750 mg kg⁻¹, p.o.)

**TABLE 2.** The effects of stevioside and preparation based on barley and brewer’s yeast extracts enriched with chromium (BBCr) on hyperglycaemic response to adrenalin load (0.2 mg kg⁻¹, s.c.) in mice

<table>
<thead>
<tr>
<th>Group II</th>
<th>Subgroup II-S (n = 6) - Stevioside 20 mg kg⁻¹ p.o. (7 days)</th>
<th>Subgroup II-BBCr (n = 6) - BBCr 750 mg kg⁻¹ p.o. (7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glycaemia (glycaemia before treatment)</td>
<td>Adrenalin load (0.2 mg kg⁻¹, s.c.) and measurement of glycaemia 45 min later (adrenalin load glycaemia before treatment)</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as X±SD. Subgroup II-S: 7-day treatment with stevioside (20 mg kg⁻¹, p.o.); Subgroup I-BBCr: 7-day treatment with BBCr (750 mg kg⁻¹, p.o.)

**FIGURE 1.** Schematic presentation of the experimental procedure.
TABLE 3. The effects of stevioside and preparation based on barley and brewer’s yeast extracts enriched with chromium (BBCr) on hyperglycaemic effects of alloxan (100 mg kg⁻¹, i.p.) in mice

<table>
<thead>
<tr>
<th>Group III</th>
<th>Glycaemia (mmol/L)</th>
<th>Before alloxan and S or BBCr treatment</th>
<th>12 days after the stevioside or BBrcr treatment and three doses of alloxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup III-S</td>
<td>n = 6</td>
<td>7.15 ± 1.22</td>
<td>14.18 ± 11.17</td>
</tr>
<tr>
<td>Subgroup III-BBBCr</td>
<td>n = 6</td>
<td>6.00 ± 0.88</td>
<td>8.63 ± 3.16</td>
</tr>
<tr>
<td>Subgroup III-placebo</td>
<td>n = 6</td>
<td>7.33 ± 0.45</td>
<td>18.77 ± 5.53</td>
</tr>
</tbody>
</table>

Results are presented as X ±SD. Subgroup III-S: 12-day treatment with stevioside (20 mg kg⁻¹, p.o.); Subgroup III-BBCr: 12 day treatment with BBcr (750 mg kg⁻¹, p.o.); Subgroup III-placebo: 12 day treatment with saline (1 ml/100g, p.o.).

There were no significant differences in fasting glycaemia (p < 0.05) between subgroup III-BBBCr and subgroup III-placebo.

DISCUSSION

Seven day treatment with stevioside resulted in statistically significant reduction of fasting glycaemia levels in group I, but did not influence fasting glycaemia levels in group II. Having in mind that glucose load (group I) and adrenaline load (group II) were done after glycaemia measuring in fasting conditions (Figure 1), fasting glycaemia values before and after treatment with stevioside, can be summarised in group I and group II (I-S + II-S; n = 12). After summarising, obtained data show that stevioside did not significantly change the fasting glycaemia levels after the 7-day treatment (7.32 ± 0.94 mmol/L vs. 7.16 ± 1.03 mmol/L; p > 0.05). This is in accordance with the mechanism of stevioside action reported by some other researchers. Jeppesen et al. [4] reported that insulinotropic effects of stevioside depend on the glucose concentration. Namely, the authors showed that stevioside (1 mmol/L) potentiated insulin secretion at the glucose concentration exceeding 8.3 mmol/L. They also observed that the insulinotropic effect was preserved in the absence of extracellular Ca²⁺ and that stevioside potentiated the insulin secretion from beta cell line INS-1 without affecting the activity of the membrane ATP-sensitive potassium channels and increase in the concentration of cyclic adenosine monophosphate (cAMP) in the islets. Ferreira et al. [16] investigated the activity of stevia leaves (20 mg kg⁻¹ day⁻¹) and stevioside (5 mg kg⁻¹ day⁻¹) on glycaemia and gluconeogenesis in rats after a 15-day application. They showed that a decrease in glycaemia and gluconeogenesis occurred after the treatment with stevia leaves but not with stevioside. To explore the effects of stevioside pre-treatment, Chen et al. [17] exposed isolated mouse islets to low or high glucose for 1 h after short-term (2 h) or long-term (24 h) pre-treatment with stevioside. Basal insulin secretion at 3.3 or 5.5 mM glucose was not changed after short-term pre-treatment with stevioside (10⁻⁷ M). Their study suggests that stevioside pre-treatment does not cause a stimulation of basal insulin secretion and does not desensitize beta-cells. In the present investigation, the glycaemia increase in the oral glucose load test was not significantly lower after the 7-day application of stevioside (I-S subgroup) compared to the values before treatment (7.85 ± 0.99 mmol/L vs. 7.73 ± 1.51 mmol/L). Lai ler et al. [18] in their study on insulin-sensitive rats showed that stevioside did not diminish the area under the curve (AUC) for glucose in the OGTT, but it diminished the AUC value for insulin by 42% and insulin index by 45%. However, there is data supporting the hypoglycaemic action of stevioside as evidenced by the OGTT. Chen et al. [17] showed that glucose stimulated insulin secretion (16.7 mM) increased dose-dependently after long-term pretreatment with stevioside at concentrations from 10⁻⁷ M to 10⁻³ M. The effect of stevioside on lowering hyperglycaemia in the OGTT was also demonstrated in healthy rats [19]. However, the 7-day treatment of mice with stevioside resulted in a statistically significant decrease of glycaemia in the adrenaline load test (II-S subgroup) compared to the values before treatment (11.65 ± 1.20 mmol/L vs. 13.45

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After the 15-day treatment with stevioside (III-S subgroup), ± 0.11 mmol/L, the mechanism of stevioside action has not been explained yet. It is thought that its oral application increases cell sensitivity to insulin in insulin-resistant rats and that it exhibits antihyperglycaemic, insulinotropic and gluconogenic static effects in the type 2 diabetic rat [20,21], the main action being insulinotropic. Stevioside also increases the utilization of glucose in the muscle tissue cells and lowers the liver gluconeogenesis [18,19]. Melis [22] reported that stevioside reduced glucose reabsorption in the kidney tubuli. Lailerd et al. [18] studied the glucose transport to the muscle tissue in vitro and found that the stevioside doses from 0.01 to 0.1 mmol/L did not change the basal transport of glucose to the muscle tissue in the insulin-resistant rats. However, at a dose of 0.1 mmol/L stevioside increased insulin-stimulated transport of glucose to m. epitrochlearis by 15% and to m. soleus by 48%. At a dose exceeding 0.5 mmol/L the effect of stevioside was reversible. Similarly, in the insulin-resistant obese rats the basal transport of glucose to muscle tissue was not affected with stevioside doses between 0.01 and 0.1 mmol/L, but these doses caused an increase of the insulin-stimulated transport of glucose to m. epitrochlearis by 15% and to m. soleus by 20%. These results indicate that glucose transport to the muscle tissue is one of the potential sites of stevioside action [18]. Regardless of the opposite opinions of the researchers concerning the mechanism of stevioside action, the majority of them agree that stevioside normalizes blood glucose level. Numerous studies reporting that stevia components showed significant decrease of glycaemia in diabetes and increase of the glycaemia in the state of hypoglycaemia justify the need for experimental work in this area [23]. In contrast to stevioside, in an analogous 7-day treatment, the BBCr produced a significant decrease of fasting glycaemia in I and II group compared to the values before treatment (I-BBCr subgroup: 8.20 ± 1.07 mmol/L vs. 7.05 ± 0.76 mmol/L, p = 0.04; II-BBCr subgroup: 7.10 ± 0.75 mmol/L vs. 6.58 ± 0.53 mmol/L, p = 0.02). These results are in accordance with the results of Miljkovic and Pietrzkowski [8], who showed that the 15-day application of a preparation based on barley extract decreased the fasting glycaemia by 12%. It is thought that the hypoglycaemic effect of BBCr is in accordance with its insulin-independent mechanism of action, consisting in a direct activation of AMPK and via the e-NOS system, increase in the expression of the Glut-4 receptor on the surface of cell membranes and enhanced takeover of glucose. After the glucose load, the glycaemia increase was statistically significant (from 7.42 ± 0.59 mmol/L vs. 9.20 ± 0.61 mmol/L, p = 0.01). Miljkovic et al. [8] showed that the 15-day application of a preparation based on barley extract yielded a decrease of postprandial glycaemia by 13%. This observation can be explained by the insulinotropic action of the preparation and increased takeover of glucose in the cells due to increased expression of the Glut-4 receptor on the cell membrane. The 7-day application of the preparation BBCr did not produce a statistically significant change of glycaemia after the adrenalin load (subgroup II-BBCr) compared to the values before treatment (14.17 ± 2.84 mmol/L vs. 15.07 ± 2.56 mmol/L). After the application of three doses of alloxa, no hyperglycaemia increase was observed in the subgroup of animals receiving BBCr (subgroup III-BBCr) for 12 days (glycaemia increased from 6.0 ± 0.88 mmol/L to 8.63 ± 3.16 mmol/L, p = 0.08). This increase in glycaemia was statistically significantly lower compared to that observed for the III-placebo subgroup (8.63 ± 3.16 mmol/L vs. 18.77 ± 5.53 mmol/L, p < 0.05). These results may be explained by the insulinotropic action of BBCr and enhanced glucose takeover in the cells, which is not insulin-dependent. Hong et al. [24] studied the action of barley extract at a dose of 62.5 mg kg⁻¹ in a 12-week treatment on glycaemia, insulinaemia and other biochemical parameters in mice with genetic diabetes. The results showed a statistically significant decrease of the glycated haemoglobin and glycaemia compared to the controls. These results were also confirmed by German researchers, who showed that a long-term application of barley and wheat proteins reduced the incidence of autoimmune diabetes [25]. Apart from the activation of AMPK, barley administration showed a beneficial effect on glycaemia due to a high content of dietetic fibres [26]. However, there is also another opinion, which ascribes the main role to chromium present in the barley at a high concentration (5.69 μg/g). The role of chromium in the control of glycaemia in rats with diabetes type 2 was studied by comparing the diets involving barley, sucrose and starch on diabetic symptoms in adult diabetic rats. The diabetes symptoms were less pronounced only in the group of animals receiving food rich in barley. The addition of trivalent inorganic chromium to sucrose in a concentration that is present in barley also caused the reduction of glycaemia and
thirst. The authors concluded that this effect may be ascribed to the high concentration of chromium [27]. It is thought that chromium accelerates the cascade of intracellular phosphorylations and enzyme activation after binding to the insulin receptors, and increases expression of the Glut-4 receptors on the surface of cell membranes [28, 9]. Bearing in mind that the preparation BBCr is enriched with organically bound chromium (28 ± 5%) the action on the translocation and the expression of the Glut-4 receptors on the cell membrane are enhanced, thus enhancing the glucose takeover, which is in agreement with the results of the present investigation.

**CONCLUSION**

BBCr caused a significant decrease of fasting glycaemia and significant reduction of glycaemia after glucose load. Stevioside caused only the decrease of adrenalin-induced hyperglycaemia. BBCr product prevented the onset of experimental diabetes in mice caused by alloxan. Based on these results it can be concluded that BBCr has its role in the prevention and treatment of hyperglycaemia in mice. A long-term human study with diabetic patients should be done to verify these promising results in animal diabetes.

**DECLARATION OF INTEREST**

Authors report no conflict of interest.

**REFERENCES**