Effect of aqueous solution of stevioside on pharmacological properties of some cardioactive drugs

Velibor Vasović*, Aleksandar Rašković*, Momir Mikov*, Ivan Mikov†, Boris Milijašević*, Saša Vukmirović*, Zorana Budakov‡

*Department of Pharmacology, Toxicology and Clinical Pharmacology, †Institute of Occupational Health, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia; ‡Department of Blood Transfusion of Vojvodina, Novi Sad, Serbia

Abstract

Background/Aim. Stevioside is a glycoside that supposedly possesses a number of pharmacodynamic effects such as anti-infective, hypoglycemic, along with the beneficial influence on the cardiovascular system. The aim of this study was to determine the effect of rats pretreatment with aqueous solution of stevioside on pharmacological actions of adrenaline, metoprolol and verapamil.

Methods. Rats were administered (intraperitoneally 200 mg/kg/day) stevioside as aqueous solution or physiological saline in the course of 5 days, then anesthetized with urethane and the first ECG recording was made. The prepared jugular vein was connected to an infusion pump with adrenaline (0.1 mg/mL), verapamil (2.5 mg/mL) or metoprolol (1 mg/mL). Control animals, pretreated with saline, in addition to the mentioned drugs, were also infused with the solution of stevioside (200 mg/mL) in the course of recording ECG.

Results. The infusion of stevioside produced no significant changes in ECG, even at a dose exceeding 1,600 mg/kg. In the control group, a dose of adrenaline of 0.07 ± 0.02 mg/kg decreased the heart rate, whereas in the stevioside-pretreated rats this occurred at a significantly higher dose (0.13 ± 0.03 mg/kg). In stevioside-pretreated rats, the amount of verapamil needed to produce the decrease in heart rate was significantly lower compared to the control. The pretreatment with stevioside caused no significant changes in the parameters registered on ECG during infusion of metoprolol.

Conclusion. The results suggest that pretreatment with stevioside may change the effect of adrenaline and verapamil on the heart rate.

Key words: stevia; phytotherapy; electrocardiography; epinephrine; metoprolol; verapamil; rats.

Correspondence to: Boris Milijašević, Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. Phone: +381 21 522 172. Fax: +381 21 6615 771. E-mail: borismed@gmail.com
Introduction

*Stevia* leaves have been used by indigenous peoples in Paraguay and Brazil since before recorded history. *Stevia* became more widely known following the 1887, when it was discovered by botanist Antonio Bertoni. Due to its sweetness, *Stevia* has been given many names including honey leaf, sweet leaf of Paraguay, sweet herb and honey herb 1. *Stevia* is used most in the countries of South America, much less in Europe, and since 1970 it has been widely used in Japan as sweetener of various beverages of mass use 2.

The major glycosides found in the leaves of wild *Stevia* plants are stevioside, rebaudioside A, rebaudioside C and dulcosides A and B 3, 4. Other sweet diterpenoid glycosides which can be isolated include rebaudioside D and E 5.

The sweet taste of *Stevia* tea is due to stevioside, a glycoside that supposedly possesses a number of pharmacodynamic effects such as anti-infective, hypoglycemic, along with the beneficial influence on the cardiovascular system and on seborrhoeic skin and skin with acne. The importance and actuality of scientific studies on *Stevia* is emphasized in many papers 6-13.

Curi et al. 14 reported that *Stevia* extracts from 5 g of dried leaves administered thrice a day for 3 days to healthy volunteers lowered the plasma glucose levels. However, care should be taken interpreting these results as the plasma glucose level of the Stevia treated group was already significantly lower before the administration of the extract 14.

Intravenous administration of stevioside [95% pure, in doses of 50, 100 or 200 mg/kg body weight (bw)] resulted in a significant hypotensive effect in spontaneously hypertensive rats without adverse effects on heart rate or serum catecholamine levels 15. In a study with humans stevioside (250 mg thrice a day) was administered for 1 year to 60 hypertensive volunteers 16. After 3 months the systolic and diastolic blood pressure significantly decreased and the effect persisted during the whole year. Blood biochemistry parameters including lipids and glucose showed no significant changes. No significant adverse effect was observed and quality of life assessment showed no deterioration. The authors concluded that stevioside is a well tolerated and effective compound that may be considered as an alternative or supplementary therapy for patients with hypertension. Liu et al. 18 reported that the underlying mechanism of the hypotensive effect of administered stevioside in dogs (200 mg/kg bw) was due to inhibition of Ca ++ influx from extracellular fluid. Also, Melis and Sainati 6 suggested that stevioside induced in rats a decrease in mean arterial pressure and promoted renal vasodilatation by lowering renal vascular resistance. The vasodilator effect is likely to occur via blockage of Ca ++ channels similarity to verapamil. Stevioside reduces blood pressure by decreasing the vascular resistance via inhibition of extracellular Ca ++ influx and by stimulating the release of a vasodilator prostaglandin. Stevioside also induces diuresis, natriuresis and reduction of Na + reabsorption resulting in the reduction of extracellular fluid volume 17.

Preliminary human studies suggest that stevioside has an influence on the function of cardiovascular system, especially that stevioside can reduce hypertension 18, 19.

The European Food Safety Authority evaluated the safety of steviol glycosides, extracted from the leaves of the *Stevia rebaudiana* Bertoni plant, as sweetener and expressed its opinion on March 10, 2010. The Authority established an acceptable daily intake for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day. On November 11, 2011, the European Commission allowed the usage of steviol glycosides as food additive, establishing maximum content levels for different types of foods and beverages 20.

The aim of this study was to determine whether stevioside can cause a significant interaction with some cardioactive drugs (adrenaline, metoprolol and verapamil) and modify their effect on the heart rate.

Methods

Stevioside in the form of white powder, purchased in Brazil (Stevita 100% Natural), was a spray-dried commercial formulation containing 95% of stevioside, purified from the *Stevia* leaves; Urethane puriss, (Reanal, Budapest, Hungary); adrenaline, (1 mg/mL, diluted to 0.1 mg/mL), Adrenaline ampoules, Jugoremedija Zrenjanin, Serbia); metoprolol, (5 mg/5 mL), Betaloc ® ampoules, Astra Zeneca UK; verapamil (5 mg/2 mL), Isopamil ampoules ®, Galenika, Belgrade, Serbia.

The experiments were carried out on adult Wistar rats of both sexes, bw 180-260 g. Before and during the experiment the animals had free access to food and water, with a 12-hour cycle of light and dark periods.

The pretreatment period lasted 5 days, during which the animals were injected intraperitoneally (ip) with daily doses of: saline solution (1 mL/kg bw) – the control group (C) or an aqueous solution of stevioside (200 mg/kg bw) – the experimental group (S).

On the 5th day, 2 h after the last injection, the animals were anaesthetized with 25% urethane and connected to the ECG, to take the initial recording. The jugular vein of animals was prepared and animals were connected to the infusion pump.

The animals from the group C were connected to the infusion pump containing one of the investigated cardioactive drugs: adrenaline (0.1 mg/mL), metoprolol (1.0 mg/mL), verapamil (2.5 mg/mL), or aqueous solution of stevioside (200 mg/mL).

The animals from the group S were connected to the infusion pump containing one of the investigated cardioactive drugs: adrenaline (0.1 mg/mL), metoprolol (1.0 mg/mL), or verapamil (2.5 mg/mL).

The infusion rate for verapamil was 0.1 mL/min, ECG was monitored 12 minutes during verapamil infusion got. The infusion rate for the other drugs was 0.2 mL/min, ECG was also monitored during 12 minutes of infusion with other drugs (adrenaline, metoprolol or aqueous solution of stevioside).

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ECG analysis was performed in a single-blinded fashion. In all of animals in the group C and the group S ECG was recorded before application of cardioactive drugs. Also the investigator who conducted the experiment did not interpret ECG. It was interpreted by the investigator who did not know the treatment.

The ECG paper speed was 25 mm/sec, respectively one small block was 40 msec. On the basis of the time interval of infusion duration and the change in the ECG it was possible to calculate the amount of the drug required to produce the observed changes. This amount was correlated with the animal’s bw to obtain the specific dose in mg/kg bw.

For all animals 5 min before the application of the investigated cardioactive drugs the control ECG was recorded. Therefore, each of the animal was a control for itself (Figure 1a).

The infusion pump and ECG machine started at the same time. During the application of cardioactive drugs changes in the ECG record were monitored.

The changes that were observed on the ECG are: 1) the first changes – lonely change in the heart rate that was seen in ECG recording. This changes in the heart rate most frequently were extrasystoles (Figure 1b) or atrioventricular block (Figure 1c); 2) second changes – frequently changes in the heart rate that were seen in the ECG recording (bradycardia was the most frequent change in the heart rate (Figure 1d); 3) the third changes or toxic effect – changes in the heart rate that were seen in the ECG recording in the form of extreme bradycardia (Figure 1e) or asystolia (cardiac arrest) (Figure 1f). Extreme bradycardia was frequency of the heart less than 100 beats per minute.

![Figure 1](image-url)

Fig. 1 – The changes that were monitored during infusion of the drugs tested.

Statistical analysis was performed by Student’s t-test for small independent samples. Values $p < 0.05$ were considered statistically significant.

This experiment was carried out in accordance with the ethical principles of working with experimental animals (AEC approval Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia).

**Results**

No toxic effect was observed in any case of stevioside infusion not even after the dose exceeding 1,600 mg/kg. ECG record of animals in the control group after infusion of stevioside was similar to the ECG record before infusion of stevioside. A decrease of frequency was observed in 2 out of 6 animals, and only after 240 s of infusion, which corresponded to the dose of 640 mg/kg. Further infusion of stevioside produced the normalization of the heart frequency after 180–300 s.

Infusion of adrenaline in all of the animals in the control group decreased the heart rate after the dose of $0.07 \pm 0.02$ mg/kg. None of the animals died but attenuated amplitudes of the QRS complex were observed in two cases after the dose of adrenaline of $0.56 \pm 0.014$ mg/kg. During infusion that lasted about 12 min, after the dose of adrenaline of 0.95 mg/kg, the changes in the ECG pattern that could indicate the occurrence of toxicity were not detected.

Pretreatment of rats with stevioside changed the sensitivity of the myocardium to adrenaline (Table 1). Namely, the decrease in the heart rate occurred significantly later compared to the control group of animals, at the doses of $0.13 \pm 0.03$ mg/kg. In this group of animals, an increased toxic effect of adrenaline was observed. Two out of 6 animals died after infusion of adrenaline of 0.82 mg/kg and 0.92 mg/kg, respectively.

The pretreatment of rats with stevioside decreased the sensitivity of the heart to adrenaline, and increased its toxicity.

The effect of verapamil on ECG of the control animals and those pretreated with stevioside is given in Table 1. The fusion of verapamil in the control group caused the first reaction at the dose of $1.84 \pm 0.38$ mg/kg, while the second reaction was caused with the dose of $3.78 \pm 0.89$ mg/kg. Toxic effects during the infusion of verapamil in the control group were registered after the dose of $7.53 \pm 1.45$ mg/kg. All of the three evaluated parameters (the first, second and the third reaction of the heart to verapamil infusion) in the stevioside-pretreated group, occurred significantly earlier comparing with the control group.

The effect of metoprolol on ECG of the control animals and those pretreated with stevioside is given in Table 1.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ECG changes</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Adrenaline (mg/kg), $\bar{x} \pm SD$</td>
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<tr>
<td>with S pretreatment</td>
<td>$0.13 \pm 0.03$*</td>
</tr>
<tr>
<td>without S pretreatment</td>
<td>$0.07 \pm 0.02$</td>
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<tr>
<td>Verapamil (mg/kg), $\bar{x} \pm SD$</td>
<td></td>
</tr>
<tr>
<td>with S pretreatment</td>
<td>$0.89 \pm 0.17$*</td>
</tr>
<tr>
<td>without S pretreatment</td>
<td>$1.84 \pm 0.38$</td>
</tr>
<tr>
<td>Metoprolol (mg/kg), $\bar{x} \pm SD$</td>
<td></td>
</tr>
<tr>
<td>with S pretreatment</td>
<td>$1.89 \pm 0.67$</td>
</tr>
<tr>
<td>without S pretreatment</td>
<td>$1.45 \pm 0.41$</td>
</tr>
</tbody>
</table>

S - stevioside-pretreated rats (200 mg/kg/day, intraperitoneally during 5 days); ECG changes: I – first visible; II – more continuous; III – toxic; $\bar{x}$ – mean value; SD – standard deviation. *$p < 0.05$ (statistically significant difference).

The present data confirm that pretreatment with stevioside play an important role in the increase or decrease of the sensitivity of the myocardium in experimental animals to the studied drugs.

The infusion of aqueous solution of stevioside produced no significant changes in ECG. Only in two cases a temporary decrease in heart rate was observed, but the initial value was restored within 3 to 3.5 min during continuous infusion of stevioside. Similar results were also observed in our first study, when the concentration of stevioside in infusion was 20 mg/mL. Chan et al. found that stevioside applied intravenously in the dose of 200 mg/kg in rats was effective in blood pressure reduction and there was no change in the heart rate. The heart rate in rats were about 350 beats per minute. Clinical studies also show that stevioside applied at a dose of 250 mg three times a day orally for one year in people with hypertension exerts an antihypertensive effect but no effect on the heart rate. The study confirms our results that stevioside at a high dose of 1,600 mg/kg did not significantly affect the heart rate.

In both cases (with control the and stevioside-pretreated rats) the decreased heart rate was observed during the adrenaline infusion. This result can be explained by the fact that the rats are laboratory animals in which α1 adrenergic receptors are very sensitive to adrenaline, resulting in the significant vasoconstriction and reflex bradycardia during infusion of adrenaline. This fact was first pointed out by Turner in 1965.

In this study, the pretreatment of rats with stevioside reduced the sensitivity of the myocardium to adrenaline (bradycardia occurred later compared to the control).

In our previous study, when the animals were pretreated with a lower dose of stevioside (20 mg/kg bw) the toxic effects (asystolia, cardiac arrest) were not reported. But, the pretreatment with a higher dose of stevioside (200 mg/kg bw) increased the sensitivity of the myocardium of rats to adrenaline, and cardiac arrest in stevioside–pretreated rats occurred after the administration of adrenaline in a dose of 0.81 ± 0.08 mg/kg bw.

The mechanism of the hypotensive action of verapamil is the blocking of calcium channels. The consequence of calcium channels blocking in the myocardium is the appearance of bradycardia. Stevioside, as reported in several papers is a calcium channels blocker. In stevioside pretreated animals, an increased sensitivity of the myocardium to verapamil was observed and the drug toxicity was significantly increased, too. Thus, a significantly smaller amount of verapamil was required to cause bradycardia in the stevioside–pretreated animals, which needed a significantly smaller amount of verapamil to cause toxic effects (cardiac arrest).

In this study, the pretreatment of rats with stevioside at a dose of 20 mg/kg bw there was no report on the toxic effects (asystolia, cardiac arrest) were not reported. But, the pretreatment with a higher dose of stevioside (200 mg/kg bw) increased the sensitivity of the myocardium of rats to adrenaline, and cardiac arrest in stevioside–pretreated rats occurred after the administration of adrenaline in a dose of 0.81 ± 0.08 mg/kg bw.

The results from Jeppesen et al. in the experiments on rats indicate that the treatment with stevioside exhibited hypoglycemic, insulintropic and glucagonostatic effects, which might influence the sensitivity of the heart to cardioactive drugs actions.

Conclusion

Our results suggest that pretreatment with stevioside might change the effects of adrenaline and verapamil on the heart rate.

Acknowledgements

This research was supported by the Grant of the Ministry of Education, Science and Technological Development of the Republic of Serbia, project No 41012. This work was also supported by the Secretariat for Science and Technological Development, Autonomous Province of Vojvodina, project No 114-451-2458/2011.

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Received on May 24, 2012.
Revised on March 7, 2013.
Accepted on March 10, 2013.