International Food Research Journal 28(2): 342 - 349 (April 2021)
Journal homepage: *http://www.ifrj.upm.edu.my*

Evaluation of minor steviol glycosides effect on insulin resistance, serum triglycerides, and antioxidant capacity of diabetized Wistar rats

[1]Diaz-Canul, C., [1]Coop-Gamas, F. Y., [1]Ávila-Escalante, M. L.,
[2]Betancur-Ancona, D. A. and [1]*Aranda-González, I. I.

[1]Autonomous University of Yucatan, Faculty of Medicine, Itzáez Av. No. 498 x 59 and 59A Col. Centro. Mérida, Yucatán, CP 97000, México
[2]Autonomous University of Yucatan, School of Chemical Engineering, Periférico Norte Kilómetro 33.5, Tablaje Catastral 13615 Chuburna de Hidalgo Inn. Mérida, Yucatán C.P. 97203, México

Article history

Received: 12 November 2019
Received in revised form: 27 October 2020
Accepted: 30 October 2020

Keywords

diabetes mellitus,
QUICKI,
SOD,
Stevia rebaudiana,
superoxide dismutase

Abstract

The objective of the present work was to evaluate the effect of minor glycosides on the QUICKI index as a marker of insulin resistance, triglycerides (TG), and antioxidant capacity in Wistar rats induced with diabetes mellitus type 2 (DM2). DM2 was induced in male Wistar rats (n = 35) through streptozotocin-nicotinamide. Hyperglycaemia was confirmed two weeks later, the subjects were divided into seven experimental groups, and each group was treated as follows: (1-5) dulcoside A, steviolbioside, rebaudioside B, C, and D (20 mg/kg, respectively); (6) metformin (180 mg/kg); and (7) standard diet, orally for four weeks. Blood sample was obtained from the tail before and after the treatment. The serum was separated after clotting by centrifugation. The included parameters namely serum triglycerides (TG) and superoxide dismutase (SOD) activity were measured before and after the treatments, then the changes were determined; and at the end of the treatment, the QUICKI index was determined. The analysis of one-way variance (ANOVA) was performed considering $p < 0.05$. No statistically significant differences were found in any of the three variables ($p > 0.05$); however, the rebaudioside group B had the highest QUICKI index, while the reduction of triglycerides was greater in rebaudioside D. SOD activity increased in all groups, but was higher in rebaudioside D and steviolbioside. Minor glycosides at the dose and time evaluated had no significant effects on QUICKI index, antioxidant capacity, and triglycerides concentration.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterised by hyperglycaemia, induced by a deficiency in insulin secretion resistance to its action at the cellular level, or both (IDF, 2015). According to the International Diabetes Federation, in 2017 the prevalence of DM in adults was approximately 425 million, and it is predicted that this amount will increase to 629 million by 2045 (IDF, 2015).

Several studies have shown that a chronic exposure to high glucose concentrations generates a pro-oxidant state that is related to the appearance of metabolic complications. Another important feature of diabetes is the presence of hyperinsulinemia that inhibits the release of fatty acids, thus leading to dyslipidaemia (Erion and Corkey, 2017). Current recommendations of the American Diabetes Association (ADA) include a change in lifestyle and drug treatment. However, there is a continuous search for alternatives that do not have the adverse effects of medications. Among these alternatives, *Stevia rebaudiana* leaves have been used, as part of traditional medicine. The total composition of steviol glycosides on a *Stevia* leaf varies depending on the species, but the most common composition is stevioside (10%), rebaudioside A (2 - 5%), rebaudioside C (1%), dulcoside A (0.5%), rebaudioside D, E, and F (0.2%, respectively), steviolbioside (0.1%), and rebaudioside B (traces), reported as percentage by weight (Signh *et al.,* 2012; Ceunen and Geuns, 2013).

Individuals with DM2 have insulin hormone resistance, which occurs when cells have lower response to their effect, and leads to higher concentrations of this hormone in trying to maintain a normal blood glucose level (Trout *et al.,* 2007). Thus, the effectiveness of insulin is reduced in lowering the concentration of blood glucose, catalysing its storage as glycogen, and decreasing hepatic gluconeogenesis (Trout *et al.,* 2007). There are several methods to assess

*Corresponding author.
Email: irma.aranda@correo.uady.mx

DOI:
https://doi.org/10.47836/ifrj.28.2.15

insulin resistance (Cacho *et al.,* 2008). The euglycemic-hyperinsulinemic clamp test is considered as the "gold standard" but it is invasive and expensive. The *Quantitative Insulin Sensitivity Check Index* (QUICKI) is inexpensive and simple, requiring only a simple plasma sample, and is valid for the use on rats and mice (Muniyappa *et al.,* 2009).

Dyslipidaemia is one of the highest risk factors in cardiovascular diseases in patients with DM (Mooradian, 2009). Hyperinsulinemia is associated with an increase in the number of VLDL and TG particles in the plasma, leading to a decrease in concentrations of high-density lipoproteins (c-HDL), and an increase in the concentration of denser particles of low-density lipoproteins (LDL) (Mooradian, 2009; Schofield *et al.,* 2016). Another characteristic of DM is the presence of oxidative stress, which is defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense system, which can cause damage to various tissues (Betteridge, 2000).

Various experimental studies have been conducted to evaluate the effect of steviol glycosides, as well as the extract of *S. rebaudiana* leaves on insulin, triglycerides, and oxidative stress. *Stevia* leaf extract lowered glucose and insulin, and also increased the activity of superoxide dismutase (SOD) and catalase (CAT) in rats (Shivanna *et al.,* 2013); while in humans with DM2, the concentration of fasting and postprandial blood glucose decreased, as well as triglycerides (Ritu and Nandini, 2016). On the other hand, stevioside administered to rodents has been shown to decrease glucose, insulin, and cholesterol (Chen *et al.,* 2005, Geeraert *et al.,* 2010), as well as an increase in the expression of the enzyme of SOD (Geeraert *et al.,* 2010). In humans, a decrease in postprandial glucose has been reported (Gregersen *et al.,* 2004). Another major glycoside, rebaudioside A, has shown conflicting result, since the administration on rats has shown a decrease in glucose and increase in plasma insulin (Saravanan *et al.,* 2012), as well as an increase in the activity of antioxidant enzymes of SOD, CAT, and glutathione peroxidase (GPx) (Saravanan and Ramachadran, 2013). However, there are also reports where there was no effect on insulin and glucose on rats (Dyrskog *et al.,* 2005) or on humans (Shin *et al.,* 2016).

The studies described above have focused on complete extracts of *Stevia* leaves and their major glycosides namely rebaudioside A, and stevioside; and there are also a few studies evaluating other glycosides. A study with the minor glycosides namely dulcoside A, steviolbioside, rebaudioside B, C, and D showed that they do not have an antihyperglycemic effect in normoglycemic or DM-induced Wistar rats (Aranda-González *et al.,* 2016); however, their effect on insulin resistance, dyslipidaemia, or antioxidant activity has not been assessed.

Therefore, the objective of the present work was to evaluate the effect of consumption of minor steviol glycosides on the insulin resistance index, the concentration of serum triglycerides, and their antioxidant capacity in an experimental model of Wistar rats induced with DM2.

Materials and methods

A total of 35 Wistar rats were acquired from the animal facility in the Regional Research Centre of the Autonomous University of Yucatan (CIR-UADY)

Reagents

The glycosides used for the tests were purchased from Anhui Minmetals (Hefei, China): dulcoside A (64432-06-0), steviolbioside (41093-60-1), rebaudioside B (58543-17-2), C (63550-99-2), and D (63279-13-0). Streptozotocin and nicotinamide were purchased from Sigma-Aldrich (St. Louis, MO, USA), as well as commercial metformin (Glucophage®).

Diabetes induction

This was carried out on male Wistar rats, weighing 200 - 250 g, after 12 h of fasting by injection of 65 mg/kg streptozotocin (STZ) dissolved in citrate phosphate buffer 0.1 M, pH 4.5, and intraperitoneally (i.p.), 15 min after the administration of 120 mg/kg nicotinamide (i.p.). Hyperglycaemia was confirmed after two weeks, and only animals with blood glucose $\geq$ 200 mg/dL were included.

Treatments

After confirmation of hyperglycaemia, the animals were randomly assigned to one of the experimental groups (n = 5): (1) Control; (2) rat group treated with dulcoside A; (3) rat group treated with steviolbioside; (4) rat group treated with rebaudioside B; (5) rat group treated with rebaudioside C; (6) rat group treated with rebaudioside D; and (7) rat group treated with metformin. The glycosides were administered at a dose of 20 mg/kg, while metformin at a dose of 180 mg/kg; and the control group received no treatment. The experiment lasted for four weeks, and the glycoside dose was determined according to previous reports where stevioside was used. The treatments were administered orally and also incorporated into animal food.

The first plasma and serum samples were

obtained a day after the confirmation of the hyperglycaemic state, and before starting the treatment. Blood was obtained after 6 h of fasting, and after anesthetizing the animal with pentobarbital (30 mg/kg, i.p.). Subsequently, a small cut was made at the tip of the animals' tail, and a drip of blood was obtained, discarding the first drop of blood and collecting the following in micro tubes previously treated with heparin (1.0 µL/ml); blood was also collected without anticoagulant, and allowed to stand for 30 min at room temperature, then centrifuged at 4,000 *g* for 15 min at 4°C, and then the serum was collected. The samples were stored at -20°C for subsequent analysis.

Serum quantitative insulin sensitivity check index (QUICKI)

The enzyme linked immunoadsorption assay (ELISA) was used with the EZRMI-13K Kit. Rat/Mouse insulin was applied using ELISA Kit© (Merck Millipore®), following the steps established by the manufacturer. The QUICKI index was calculated using Eq. 1 (Trout *et al.,* 2007; La-Corte *et al.,* 2008):

$$\text{QUICKI index} = 1/(\log I_0 + \log G_0) \quad \text{(Eq. 1)}$$

where, I_0 = fasting plasma insulin (µU/mL) (1 µ U/mL= 6,945 pmol/L) (Lutz, 2010), and G_0 = fasting plasma glucose (mg/dL).

Serum triglycerides

The amount of serum triglycerides in blood serum was determined quantitatively before and after the four weeks of treatment, by reflectance photometry using the Accutrend® Plus meter following the steps established by the manufacturer. The variation in the concentration of triglycerides in each rat was calculated paired way using Eq. 2:

$$\Delta TG = TG_{Final} - TG_{Initial} \quad \text{(Eq. 2)}$$

where each variable represented the same organism before and after treatment.

Plasma superoxide dismutase activity

The activity of enzyme SOD was measured in blood plasma before and after four weeks of treatment following the method described by Yi *et al.* (1988) who used nitro blue tetrazolium (NBT) chloride to calculate the concentration of superoxide radicals generated by xanthine oxidase and hypoxanthine. The results were expressed in units of enzymatic activity (UEA), where one standard unit of enzyme activity is defined as the amount of SOD that produces 50% inhibition of NBT. Percent inhibition and enzyme activity units were calculated using Eqs. 3 and 4 (Yi *et al.,* 1988; McCord, 2001):

$$\%\ \text{Inhibition} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100\% \quad \text{(Eq. 3)}$$

$$\text{Units of enzymatic activity} = \%\ \text{Inhibition} / (100 - \%\ \text{Inhibition}) \quad \text{(Eq. 4)}$$

Ethical considerations

Animal management was performed according to the guidelines for care and use of experimental animals of the European Communities Council Directive 86/609/EEC and the "Official Mexican Standard NOM-062-ZOO-100-1999, technical specifications for the production, care and use of laboratory animals" (Diario Oficial de la Federación, 2001). Likewise, the project was approved by the Committee for the Care and Use of Laboratory Animals (CCUAL-FM-UAEM) on January 16, 2014 with the authorization number 003/2014. The animals were housed individually under controlled temperature conditions (24°C), light-dark cycles (12/12 h), and with food and water *ad libitum*.

Statistical analysis

The results were expressed as mean ± standard deviation (SD), and the difference between groups was analysed by applying the statistical test of variance analysis (ANOVA), with a 95% confidence interval and value $p \leq 0.05$, using the GraphPad Prism v.6 statistical package.

Results and discussion

The concentration of insulin and glucose are shown in Table 1. As it can be observed, the concentration of insulin was lower in the group treated with dulcoside A, whereas higher in the group treated with rebaudioside C, followed by steviolbioside. In the case of glucose, the lowest concentration was found in both of the groups which received metformin and no treatment (control). Statistical analysis showed that there were no significant differences in the concentrations of glucose (p = 0.100) and insulin (p = 0.247), as compared to the control group.

The QUICKI index for each group was calculated using the results mentioned above, obtaining similar values between the control group (0.285) and the treatments. Treatment with

Table 1. Concentration of insulin and serum glucose in rats with induced DM after treatment.

Treatment (*n* = 5 each)	Insulin (ng/mL)	Glucose (mg/dL)
Control	0.383 ± 0.200	308.00 ± 93.09
Dulcoside A	0.302 ± 0.202	366.25 ± 59.20
Steviolbioside	0.628 ± 0.414	395.50 ± 48.78
Rebaudioside B	0.561 ± 0.253	364.25 ± 41.29
Rebaudioside C	0.699 ± 0.287	424.80 ± 52.16
Rebaudioside D	0.346 ± 0.350	362.00 ± 37.31
Metformin	0.570 ± 0.293	457.20 ± 68.59

Values are mean ± standard deviation. No significant differences ($p > 0.05$) with respect to the control group (ANOVA).

rebaudioside B (0.278) presented the highest index, while the groups treated with dulcoside A (0.273) and rebaudioside D (0.273) obtained the lowest, below the metformin (0.274), rebaudioside C (0.275), and steviolbioside (0.276) groups, as seen in Figure 1. No significant differences were found in the treatments with respect to the control ($p = 0.57$).

Under normal conditions, an increase in glucose concentration acts as the main secretagogue in the release of insulin (Wilcox, 2005; Trout *et al.,* 2007). Although there are several models of DM2 in rats, the present work used the application of STZ-nicotinamide which induces 60% loss of β cells function and insulin deficiency, but not insulin resistance (Furman, 2015).

The administration of STZ-nicotinamide causes a decrease in the concentration of insulin that increases blood glucose (Szkudelski, 2012; Koksal, 2015). Therefore, tendency to increase the insulin concentration of the groups treated with steviolbioside and rebaudioside C could be caused by stimulation of the β cells acting as secretagogues, as reported with the major glycosides, rebaudioside A and stevioside, which had insulinotropic action on Wistar rats with induced DM and Goto-Kakizaki rats (Suanarunsawat and Chaiyabutr, 1997; Dyrskog *et al.,* 2005; Geeraert *et al.,* 2010). However, it should be mentioned that in the first study, the dose was much higher (200 mg/kg), and in the other two, a lower dose was used but for a longer period of time as compared to the present work. On the other hand, the insulinotropic effect reported for the *Stevia* leaf extract could be due to the glycoside content (mainly the major ones) as well as the polyphenols present, as previously reported (Shivanna *et al.,* 2013).

However, since glycaemia is a dynamic process, measuring insulin sensitivity is more adequate than just glucose or insulin. A negative correlation between insulin and the index has been described, which suggests that an increase in insulin concentration is associated with a decrease in tissue sensitivity (Lasram *et al.,* 2014). Therefore, the QUICKI index increases as the insulin concentration decreases, an effect studied on rats diabetized with STZ and on humans with type 2 DM (Hauache and Vieira, 2003; Barman and Srinivasan, 2016). The decrease in the QUICKI index represents a reduction in insulin sensitivity, and consequently, a resistance to its action (Sasidharan *et al.,* 2013). In the present work, although no significant differences were found, glycoside treatments obtained lower QUICKI values than the control group. This can be explained in those

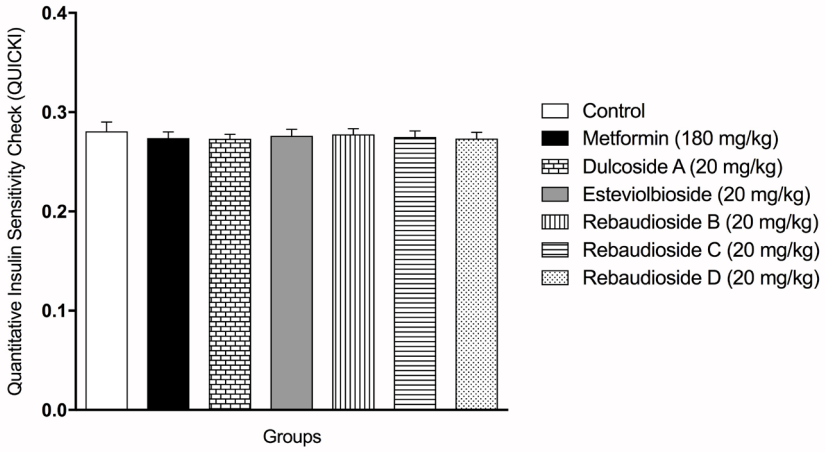

Figure 1. QUICKI index determined in serum of rats with induced DM after treatment of four weeks ($n = 5$). The QUICKI index was calculated using Eq. 1. The analysis between groups was performed using one-way ANOVA test. Columns are mean with bars indicating standard error of the mean. No significant differences ($p > 0.05$) with respect to the control group (ANOVA).

potential secretagogues, where a greater amount of insulin decreases the QUICKI index, which would be reflected on a reduction in sensitivity.

Metformin, a medication widely used in DM2 which exerts an antihyperglycemic effect (Castro-Serna and Castro-Martínez, 2006; Cacho *et al.,* 2008; Saravanan *et al.,* 2012; Schofield *et al.,* 2016; ADA, 2017a; 2017b), did not improve the concentrations of glucose, insulin, and therefore, the QUICKI index. This may be due to the fact that antihyperglycemic effect of metformin depends on the dose administered; it requires high concentrations to exert an effect on peripheral tissues, and also, the mechanism is slower in these tissues (Castro-Serna and Castro-Martínez, 2006; Cacho *et al.,* 2008), where the effect on the index was observed after four weeks of treatment (Roden *et al.,* 2005; La-Corte *et al.,* 2008). For that reason, it is likely that the dose and time of the administration of metformin treatment in the present work were insufficient to exert an effect on the concentration of insulin, glucose, and consequently on insulin sensitivity. This means that it cannot be ruled out as a higher dose of glycosides or a longer period of time could have a significant effect.

The serum triglyceride concentration was determined before and after four weeks of treatment, and is shown in Table 2. The triglyceride concentration of the group that received rebaudioside D after four weeks of treatment significantly decreased as compared to the group treated with metformin ($p \leq 0.05$).

Since the concentration of triglycerides is dynamic, the variation was calculated on the concentration of TG in each paired rat following Eq. 2. As observed in Figure 2, all the groups presented a decrease in the TG concentration, where the decrease was greater in the groups treated with rebaudioside D (-207 mg/dL) and dulcoside A (-127.25 mg/dL), while the decrease was smaller with treatments rebaudioside C (-79 mg/dL), steviolbioside (-43 mg/dL), rebaudioside B (-47.25 mg/dL), and

Table 2. Serum triglyceride concentration in rats with induced DM, before and after treatment.

Treatment (n = 5 each)	**Triglycerides (mg/dL)**	
	Pre-treatment	**Post-treatment**
Control	577.6 ± 50	473.40 ± 79.3[a]
Dulcoside A	600 ± 40	472.80 ± 110.9[a]
Steviolbioside	557 ± 96	514 ± 122.2[a]
Rebaudioside B	460.25 ± 161.8	413 ± 141.4[a]
Rebaudioside C	581.60 ± 41.1	502.60 ± 94.3[a]
Rebaudioside D	507.20 ± 135.1	363.20 ± 148.8[a#]
Metformin	596.16 ± 9.39	580.33 ± 48.1[a]

Values are mean ± standard deviation. Different letters denote significant difference before and after treatment using Student's *t*-test, whereas [#] denotes significant differences with respect to metformin group using ANOVA, followed by Tukey's *post hoc* test ($p < 0.05$).

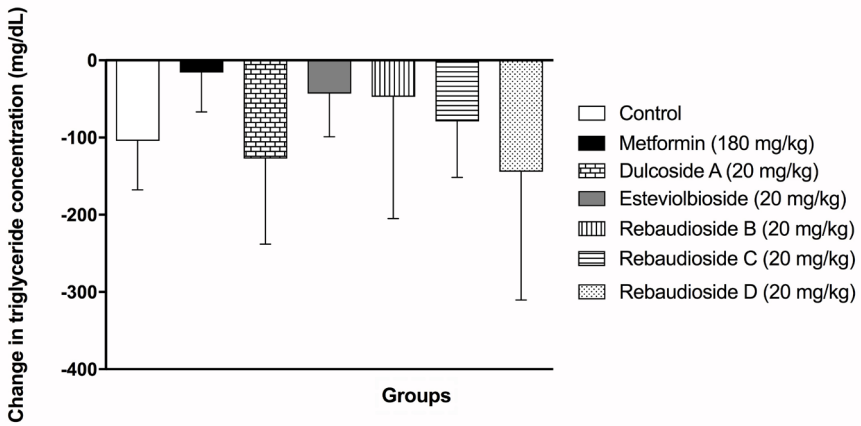

Figure 2. Variation in the concentration of serum triglycerides of the experimental groups (n = 5) before and after treatment for four weeks, calculated using Eq. 2. Columns are mean with bars indicating standard error of the mean. No significant differences ($p > 0.05$) with respect to the control group using one-way ANOVA.

metformin (-15.83 mg/dL), as compared to the control group (-104.20 mg/dL). However, no statistically significant differences (p = 0.35) were observed between the groups studied.

In DM2, hypertriglyceridemia is caused by the inactivation of the enzyme lipoprotein lipase caused by insulin resistance (Molehin *et al.,* 2018). It has been reported that the leaf extract of *S. rebaudiana* may decrease hypertriglyceridemia and glycaemia, which may be related to lower insulin resistance (Ritu and Nandini, 2016).

Dyrskog *et al.* (2005) in experiments with Goto-Kakizaki rats found no effect of major glycoside, rebaudioside A, on triglyceride concentration, unlike Saravanan and Ramachadran (2013) who demonstrated significant reduction of triglycerides using a dose of 200 mg/kg. It should be mentioned that Dyrskog *et al.* (2005) used a dose (25 mg/kg) similar to the one in the present work (20 mg/kg) with twice the time, while Saravanan and Ramachadran (2013) used a dose 10 times higher, and for 45 days.

Among the glycosides evaluated, glycoside rebaudioside D could be of interest since it yielded the greatest difference in the reduction of TG concentration, as well as the lower concentrations of glucose and insulin during four weeks of treatment, acting as the main glycoside with a hypolipemic tendency. Therefore, it is likely that a higher dose, as well as a longer period, may have a significant result.

In the results of the plasma measurement of the enzyme activity of SOD, some samples corresponding to the pre-treatment were excluded because they had haemolysis or had insufficient volume; due to this, the average SOD activity of each group was determined (n = 3 or 4). The average ($xSOD_0$) of each group was considered as the initial value, and the variation in SOD activity was calculated using Eq. 5:

$$\Delta SOD = SOD_{Final} - xSOD_{Initial} \qquad \text{(Eq. 5)}$$

Figure 3 shows that all groups presented an increase in SOD activity, where the greatest increase occurred with rebaudioside D (0.347), steviolbioside (0.300), and rebaudioside C (0.262) treatments, while the minor was found in dulcoside A (0.117); the treatments that obtained values similar to the control group were metformin (0.209) and rebaudioside B (0.211). Statistical analysis did not show significant differences (p = 0.19) between the groups.

The increase in antioxidant activity is related to the improvement in plasma insulin concentration, which in turn, improves glucose consumption in peripheral tissues, thus preventing its high concentrations and inducing oxidative stress through various mechanisms (Cefalu, 2001; ADA, 2017a; 2017b). It was found that all the experimental groups had an increase in SOD activity, where those which received glycosides had greater increase.

This increase in the antioxidant defence mechanism coincides with the reports of Saravanan and Ramachadran (2013) who concluded that the administration of rebaudioside A improved antioxidant enzyme activity and reduced oxidative stress. Another study conducted on DKO mice by Geeraert *et al.* (2010) also found that oral administration of stevioside at a dose of 10 mg/kg/day, after 12 weeks, increased the SOD activity. In both cases, the

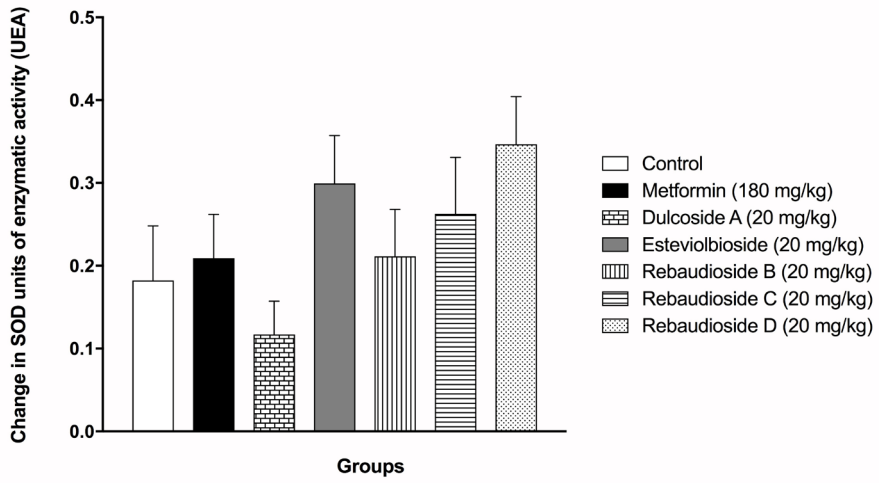

Figure 3. Variation of the SOD units of the experimental groups before and after treatment for 4 weeks (n = 5), calculated following Eq. 5. The analysis between groups was performed using one-way ANOVA test and no significant differences (p > 0.05) with respect to the control were found. Columns are mean with bars indicating standard error of the mean.

improvement in the antioxidant defence system was attributed to an increase in SOD expression. However, since both, the control group and the one treated with metformin, had an increase in SOD activity, it is not ruled out that it may be a physiological adaptation mechanism to the induced diabetes model. Considering that glycoside rebaudioside D had a greater effect on the activity of SOD and triglycerides, it represents an area of interest for future studies, where a higher dose or treatment time must be considered.

Conclusion

The treatment of diabetic rats with steviol glycosides namely dulcoside A, steviolbioside, rebaudioside B and C, given at a dose of 20 mg/kg each, for four weeks, had no significant effects on insulin resistance, antioxidant capacity, QUICKI index, and superoxide dismutase activity, respectively, nor on serum triglyceride concentration.

Acknowledgements

The authors are grateful for the support received from the Academic Development Support Program (PADECCA 2017) for the completion and publication of the present work.

We thank specially Erick Padilla Lizama and David Humberto Novelo Castañeda for their contribution in reviewing the translation of this paper.

References

American Diabetes Association (ADA). 2017a. Lifestyle management. Diabetes Care 40 (Suppl 1): S33-S43.

American Diabetes Association (ADA). 2017b. Pharmacologic approaches to glycemic treatment. Diabetes Care 40 (Suppl 1): S64-S74.

Aranda-González, I., Moguel-Ordóñez, Y., Chel-Guerrero, L., Segura-Campos, M. and Betancur-Ancona, D. 2016. Evaluation of the antihyperglycemic effect of minor steviol glycosides in normaglycemic and induced-diabetic Wistar rats. Journal of Medical Food 19(9): 844-852.

Barman, S. and Srinivasan, K. 2016. Zinc supplementation alleviates hyperglycemia and associated metabolic abnormalities in streptozotocin-induced diabetic rats. Canadian Journal of Physiology and Pharmacology 94: 1356-1365.

Betteridge, D. J. 2000. What is oxidative stress? Metabolism 49(2): 3-8.

Cacho, J., Sevillano, J., Castro, J., Herrera, E. and Ramos, M. P. 2008. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. American Journal of Physiology - Endocrinology and Metabolism 295(5): E1269-E1276.

Castro-Serna, D. and Castro-Martínez, M. G. 2006. Biguanidas. Medicina Interna de Mexico 22: 439-449.

Cefalu, W. T. 2001. Insulin resistance: cellular and clinical concepts. Experimental Biology and Medicine 226(1): 13-26

Ceunen, S. and Geuns, J. M. 2013. Steviol glycosides: chemical diversity, metabolism, and function. Journal of Natural Products 76(6): 1201-1228.

Chen, T. H., Chen, S. C., Chan, P., Chu, Y. L., Yang, H. Y. and Cheng, J. R. 2005. Mechanism of the hypoglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*. Planta Medica 71(2): 108-113.

Diario Oficial de la Federación. 2001. NOM-062-100-1999 - Technical specifications for the care and use of laboratory animals. México: Diario Oficial de la Federación.

Dyrskog, S. E., Jeppesen, P.B., Chen, J., Christensen, L. P. and Hermansen, K. 2005. The diterpene glycoside, rebaudioside A, does not improve glycemic control or affect blood pressure after eight weeks treatment in the Goto-Kakizaki rat. Review of Diabetic Studies 2(2): 84-91.

Erion, K. A. and Corkey, B. E. 2017. Hiperinsulinemia: a cause of obesity? Current Obesity Report 6: 178-186.

Furman, B. 2015. Streptozotocin-induced diabetic models in mice and rats. Current Protocols in Pharmacology 70(1): 5.47.1-5.47.20.

Geeraert, B., Crombe, F., Hulsmans, M., Benhabiles, N., Geuns, J. M. and Holvoel, P. 2010. Stevioside inhibits atherosclerosis by improving insulin signaling and antioxidant defense in obese insulin-resistant mice. International Journal of Obesity 34: 569-577.

Gregersen, S., Jeppense, P. B., Holst, J. J. and Hermansen, K. 2004. Antihyplerglycemic effects of stevioside in type 2 diabetic subjects. Metabolism 53(1): 73-76.

Hauache, O. M. and Vieira, J. G. H. 2003. Fasting insulin concentration is highly correlated with quantitative insulin sensitivity check index. Endocrine 21(2): 137-138.

International Diabetes Federation (IDF). 2015. IDF diabetes atlas. 7th ed. Belgium: IDF.

Koksal, B. 2015. Effect of streptozotocin on plasma insulin levels of rats and mice: a meta-analysis study. Open Access Macedonian Journal of Medical Sciences 3(3): 380-383.

La-Corte A., Ángel, J., Villegas, E., Bendezu, H., Ortegano, M. and Vásquez-Ricciardi, L. 2008. Insulin sensitivity indices (HOMA and QUICKI) in healthy schoolchildren and adolescents in Valera, Trujillo state, Venezuela. Venezuelan Archives of Childcare and Pediatrics 7(3): 74-78.

Lasram, M. M., Bouzid, K., Douib, I. B., Annabi, A., Elj, N. E., Fazaa, S. E., ... and Gharbi, N. 2014. Lipid metabolism disturbances contribute to insulin resistance and decrease insulin sensitivity by malathion exposure in Wistar rat. Drugs and Chemical Toxicology 38(2): 227-234.

Lutz, H. 2010. Insulin assay standardization: leading to measures of insulin sensitivity and secretion for practical clinical care. Diabetes Care 33(6): 205-206.

McCord, J. M. 2001. Analysis of superoxide dismutase activity. Current Protocols in Toxicology 1: 7.3.1-7.3.9.

Molehin, O. R., Oloyede, O. I. and Adefegha, S. A. 2018. Streptozotocin- induced diabetes in rats: effects of White Butterfly (*Clerodendrum volubile*) leaves on blood glucose levels, lipid profile and antioxidant status. Toxicology Mechanisms and Methods 28(8): 573-586.

Mooradian, A. 2009. Dyslipidemia in type 2 diabetes mellitus. Nature Clinical Practice Endocrinology and Metabolism 5(3): 150-159.

Muniyappa, R., Chen, H., Muzumdar, R. H., Einstein, F. H., Yan, X., Yue, L. Q., ... and Quon M. J. 2009. Comparison between surrogate indexes of insulin sensitivity/resistance and hyperinsulinemic euglycemic clamp in rats. American Journal of Physiology - Endocrinology and Metabolism 297: 1223-1229.

Ritu, M. and Nandini, J. 2016. Nutritional composition of *Stevia rebaudiana*, a sweet herb, and its hypoglycaemic and hypolipidaemic effect on patients with non-insulin dependent diabetes mellitus. Journal of the Science and Food Agriculture 96(12): 4231-4234.

Roden, M., Laaks, M., Widels, J. M., Urquhart, R., Richardson, C., Mariz, D. and Tan, M. H. 2005. Long-term effects of pioglitazone and metformin on insulin sensitivity in patients with type 2 diabetes mellitus. Diabetic Medicine 22: 1101-1106.

Saravanan, R. and Ramachadran, V. 2013. Modulating efficacy of rebaudioside A, a diterpenoid on antioxidant and circulatory lipids experimental diabetic rats. Environmental Toxicology and Pharmacology 36: 472-483.

Saravanan, R., Babu, K. V. and Ramachandran, V. 2012. Effect of rebaudioside A, a diterpenoid onglucose homeostasis in STZ-induced diabetic rats. Journal of Physiology and Biochemistry 68(3): 421-431.

Sasidharan, S. R., Joseph, J. A., Anandakumar, S., Venkatesan, V., Madhavan, C. N. A. and Agarwal, A. 2013. An experimental approach for selecting appropriate rodent diets for research studies on metabolic disorders. BioMed Research International 2013: article ID 752870.

Schofield, J. D., Liu, Y., Eao-Balakrishna, P., Malik, R. A. and Soran, H. 2016. Diabetes dyslipidemia. Diabetes Therapy 7(2): 203-219.

Shin, D. H., Lee, J. H., Kang, M. S., Kim, T. H., Jeong, S. J., Kim, C. H., ... and Kim, I. J. 2016. Glycemic effects of rebaudioside A and erythritol in people with glucose intolerance. Diabetes and Metabolism Journal 40(4): 283-289.

Shivanna, N., Naika, M., Khanum, F. and Kaul, V. 2013. Antioxidant, antidiabetic and renal protective properties of *Stevia rebaudiana*. Journal of Diabetes and its Complications 27(2): 103-113.

Signh, S., Garg, V., Yadav, D. and Sharma, N. 2012. *In-vitro* antioxidative and antibacterial activities of various parts of *Stevia rebaudiana* (Bertoni). International Journal of Pharmacy Pharmaceutical Sciences 4(3): 468-473.

Suanarunsawat, T. and Chaiyabutr, N. 1997. The effect of stevioside on glucose metabolism in rat. Canadian Journal of Physiology and Pharmacology 75(8): 976-982.

Szkudelski, T. 2012. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Experimental Biology and Medicine 237: 481-490.

Trout, K. K., Homko, C. and Tkacks, N. C. 2007. Methods of measuring insulin sensitivity. Biological Research for Nursing 8(4): 305-318.

Wilcox, G. 2005. Insulin and insulin resistance. Clinical Biochemist Reviews 26: 19-39.

Yi, S., Oberley, L. W. and Li, Y. 1988. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry 34(3): 497-500.