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Booklets

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Title: Effect of the consumption of Stevia rebaudiana Bertoni as a natural and artificial sweetener on fatigue and oxidative stress of skeletal

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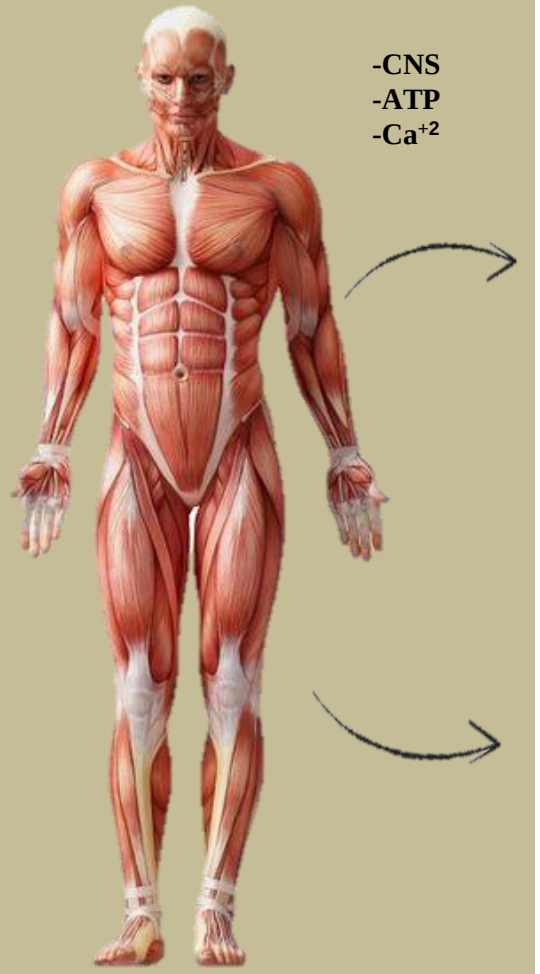
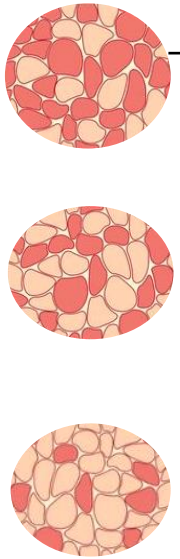
Introduction

Skeletal muscle

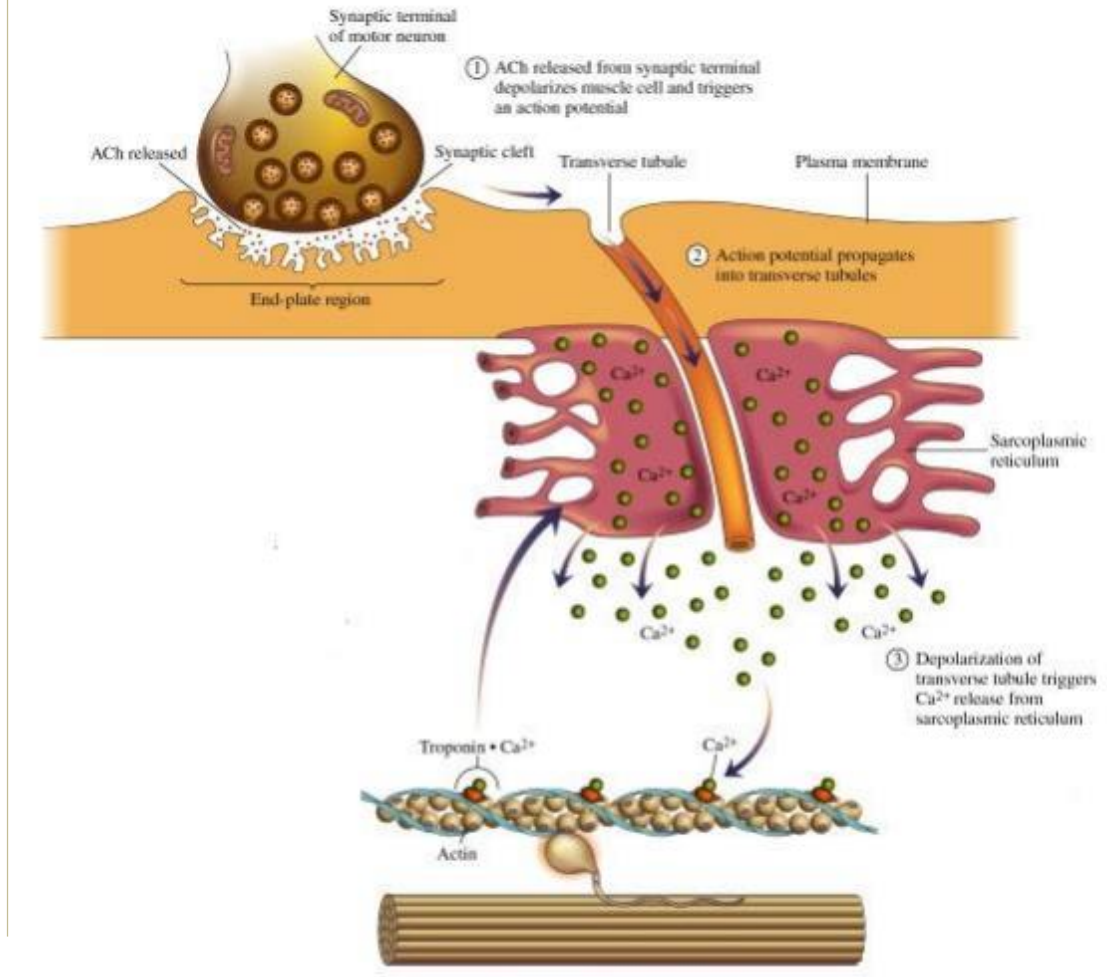


-CNS
-ATP
-Ca^{+2}

- Type l fibers
- Type lla fibers
- Type llb fibers



Skeletal muscle contraction

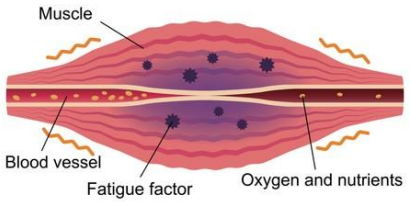


(Koeppen y Stanton, 2009; Guyton y Hall, 2016).

Muscular fatigue

Chronic diseases

Muscular fatigue



Changes

- Isometric force
- The maximum speed of shortening
- The curvature of the force-velocity relationship

Mechanisms

- Intracellular lactate and hydrogen ions
- (+) Inorganic phosphate (Pi)
- Ionic changes in PA
- Ca ++ ions of the sarcoplasmic reticulum
- Ionic changes pump Na + K + ATPase
- Depletion of phosphocreatine, glycogen, and other nutrients
- Insufficient oxygen
- Reactive oxygen species (ROS)

Main sources of ROS production

-Mitochondria -NADPH oxidase

Low ↓ **ROS** ↑ High

Optimize maximum force production

-Damage to skeletal muscle
-Contribute to the fatigue process
-Harming your recovery

Clues

- Reduced ability to withstand fatigue
- Contractile weakness
- Changes in the type of muscle fiber.
- Reduction of oxidative activity
- Peripheral insulin resistance

(Allen *et al*. 2008; Fernández *et al*. 2009).

Sweeteners consumption

Saccharose

100 g → 387 calories

Health benefits

Recommended free sugar intake (WHO)

- 10% of total caloric intake = 50g per day
- 5% of total caloric intake = 25 g per day

* 2000 kcal diet

VS

Current sugar intake

16% of total caloric intake = 80 g per day

- Obesity
- Diabetes
- Metabolic syndrome

Alternative

Sweeteners

Characteristics

- Sweet taste
- High caloric value and low (zero) caloric value
- Sweetening power (SP) : sucrose (g) / L = 1 g sweetener / L

Safety

In 1/100 of the NOAEL

No Observed Adverse Effects Level (NOAEL)

Acceptable Daily Intake (ADI) .- (additive) * (mg / kg) daily / lifetime consumption

Controversy

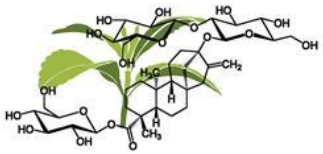
- Insulin disorders
- Acute headaches
- Tissue damage
- Weight gain and metabolic disorders
- Alter the intestinal microflora
- Imbalance in the antioxidant / pro-oxidant system
- Intracellular increase in ROS.

(OMS, 2015; AND, 2012; Echavarría y Velasco, 2012).

Stevia rebaudiana Bertoni

SP= 300

ADI = 4 mg/kg per day



Components

Steviol Glycosides
Stevioside
Steviolbioside
Isosteviol
Rebaudiosides (A, B, C, D, E, F, R and S)
Dulcosides
Steviol biosides

Phytochemical examination	
Tannins	Sterols
Alkaloids	Triterpenes
Glycosides	Anthraquinones
Phenols	Reducing compounds
Saponins	

- Control insulin levels (González, 2011).
- Anti-hypertensive (Lee et al. 2001).
- Contractile dysfunctions in the soleus muscle (El-Mesallamy et al. 2018).
- Whey protein sweetened with S. rebaudiana on muscle performance (Lima et al. 2019).

(Stephens-Camacho *et al.* 2018).

Methodology

24 rats ♂ Wistar
300-320 g
8 weeks

N=6

Official Mexican Standard NOM062-ZOO-1999

Bioethics and Biosafety Committee of the Institute of Chemical-Biological Research of the UMSNH

- Control — C —  — Conventional drinking water
- Saccharose — S —  — Cane sugar (41.2 g / L)
- Artificial *Stevia rebaudiana* — SRA —  — Formula: (6 g / L)
  -Saccharose
  -Steviol glycosides - 25mg
  -Isomalt- 10 mg
  -Sucralose -6 mg
- Natural *Stevia rebaudiana* — SRN —  — Powdered leaves of *Stevia* sp. (4.4 g / L)

In vitro tension recording

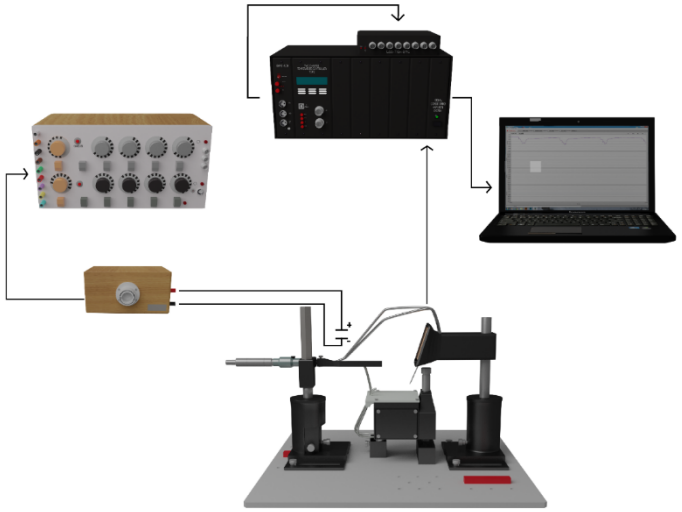


Figure 1. In vitro tension record. Series of devices interconnected to each other for the production of muscle tension recording.

Dissection

- Long Digitorum Extensor (EDL)
- Soleus

Fatigue protocol

- EDL.- 100 V, 300 ms, 50 Hz
- Soleus.- 100 V, 300 ms; 45 Hz

Biochemical tests

Measurement of levels of reactive oxygen species

- 2 ', 7'-dichlorodihydrofluorescein diacetate (H2DCFDA)
- 485 nm / 520 nm.
- Shimadzu RF-5301PC spectrophotometer (Shimadzu, Kyoto, Japan).

Catalase activity

$$H_2O_2 \rightarrow H_2O + O_2$$

Clark type oxygen electrode connected to a biological oxygen monitor (5300A Biological Oxygen Monitor, YSI, Ohio, USA).

Statistical analysis

The results obtained were analyzed by a 1-way ANOVA with Tukey's post-hoc test. Statistically significant differences were defined as $P < 0.05$.

Results

Effect of Sucrose, Artificial *Stevia rebaudiana* and Natural *Stevia rebaudiana* Sweeteners on Body Weight and Postprandial Blood Glucose

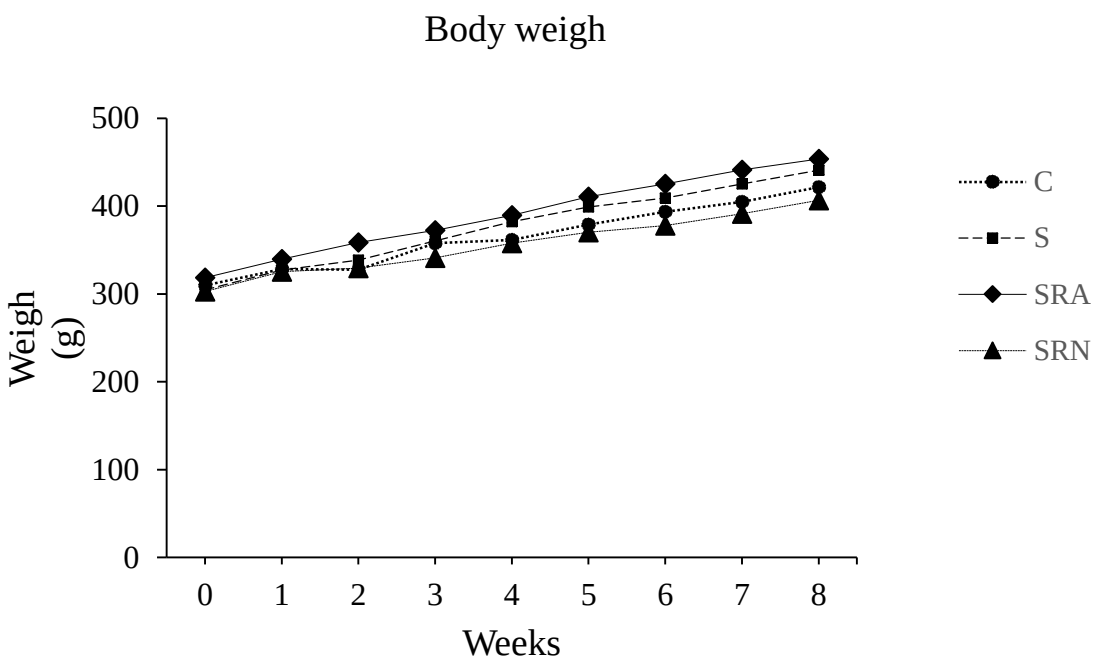


Fig. 2 Body weight. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Stevia rebaudiana natura. C (421.7 ± 51.17), SRA (453.5 ± 57.91 g), S (441 ± 17.24) and SRN (406.5 ± 39.90), l. n = 6; Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

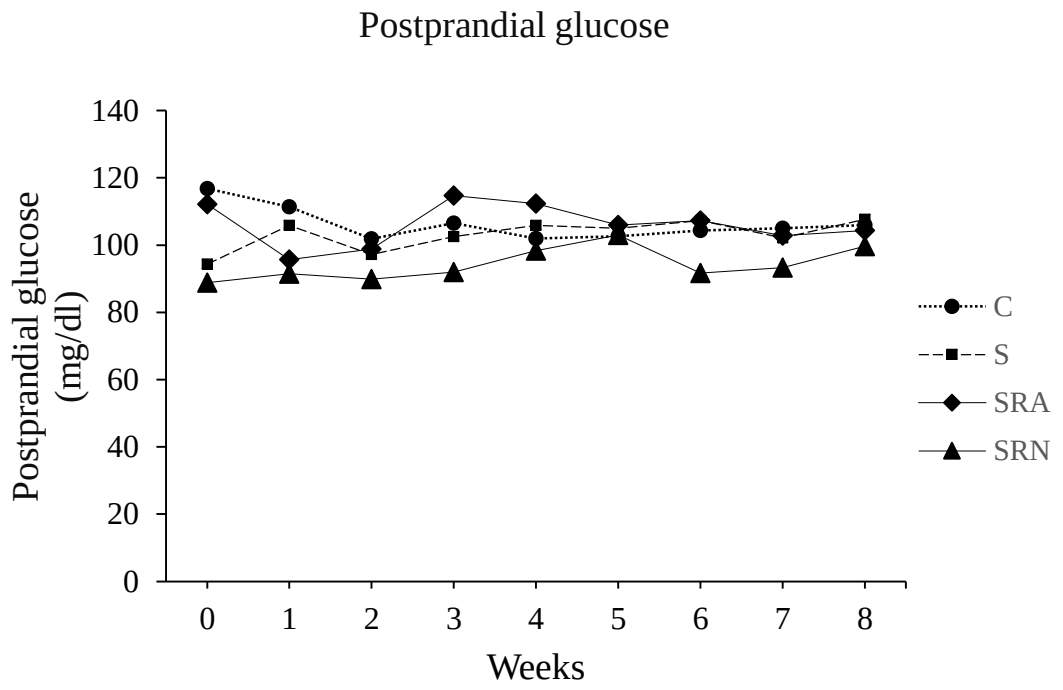


Fig. 3. Postprandial glucose. Throughout the 8 weeks of treatment with the oral administration of sweetening solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana. n = 6; Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

Effect of the sweeteners sucrose, artificial *Stevia rebaudiana* and natural *Stevia rebaudiana* on food intake

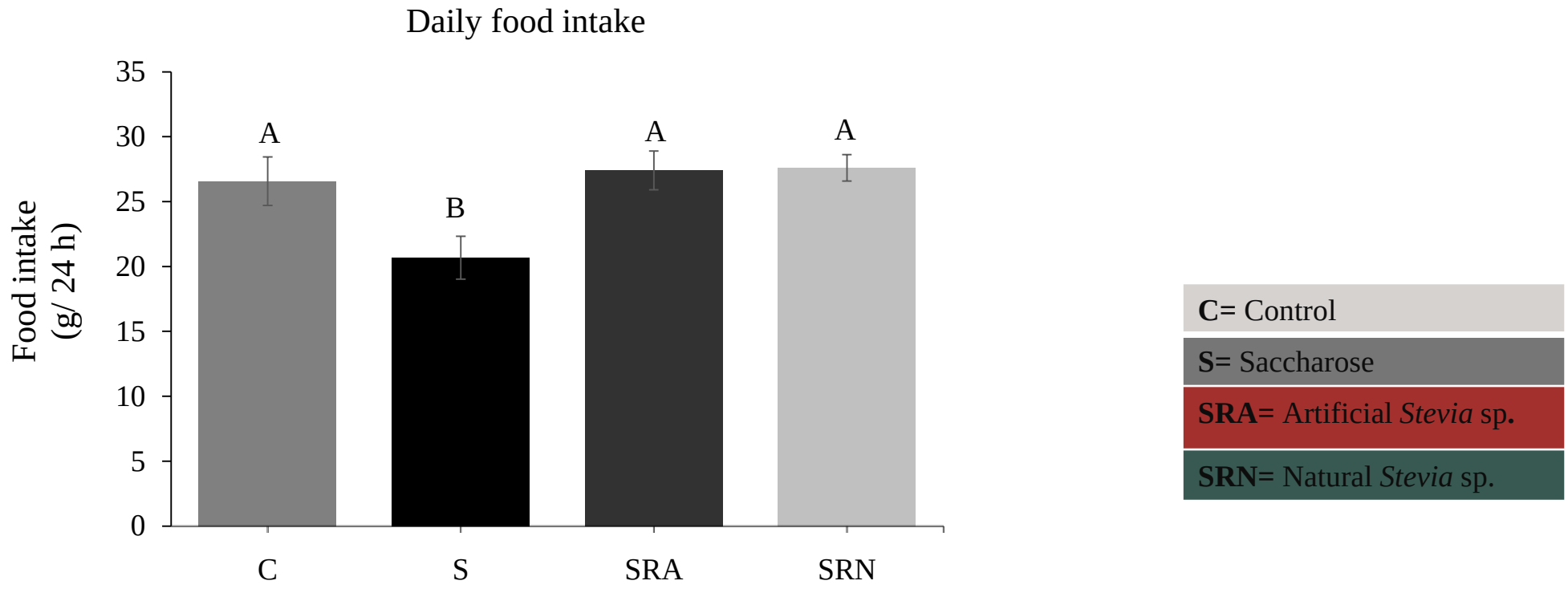


Fig. 4. Food intake. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

Effect of Sucrose, Artificial *Stevia rebaudiana* and Natural *Stevia rebaudiana* Sweeteners on Skeletal Muscle Fatigue Resistance Time

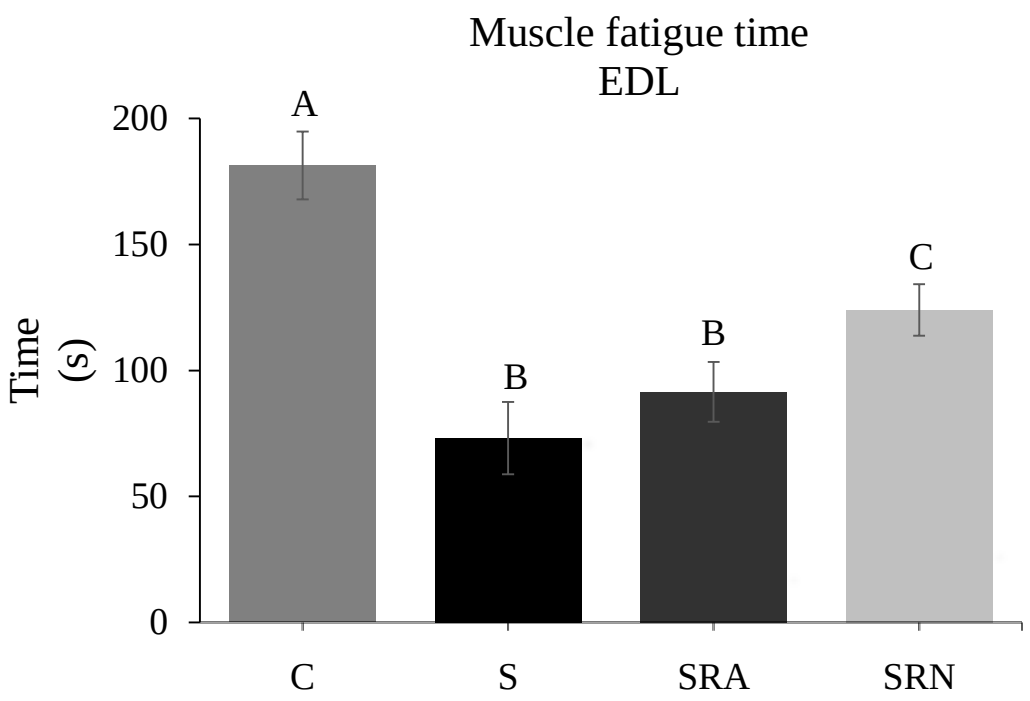


Fig. 5. Fatigue resistance time in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

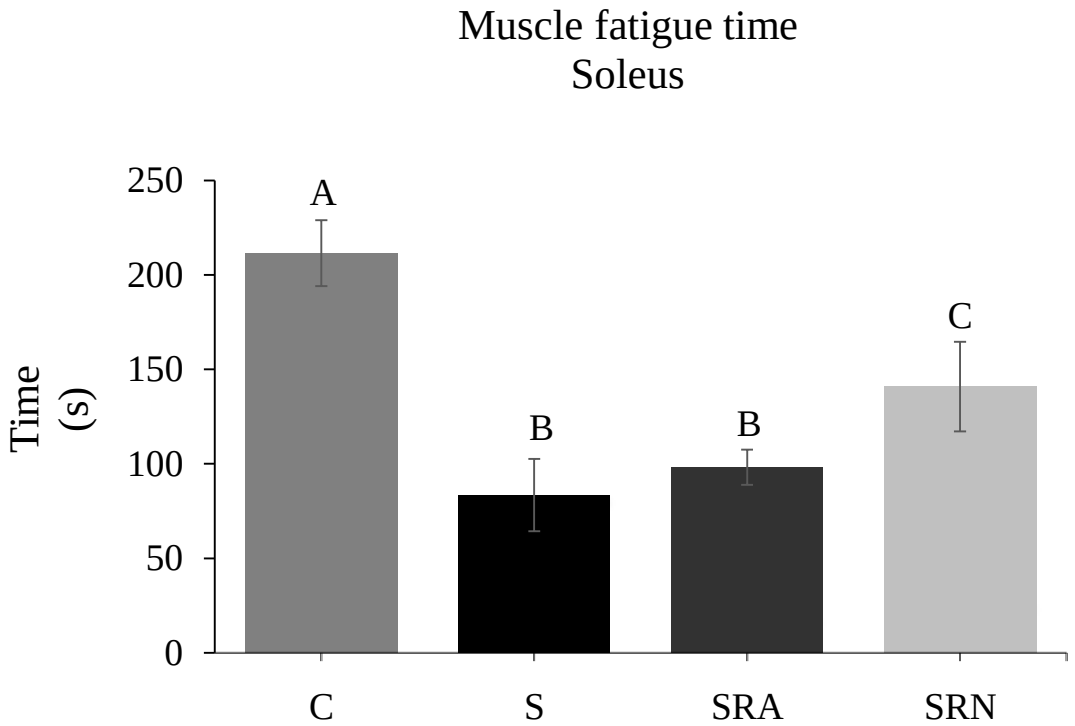


Fig. 6. Fatigue resistance time in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

Effect of Sucrose Sweeteners, Artificial *Stevia rebaudiana* and Natural *Stevia rebaudiana* on Maximum Tension and Total Skeletal Muscle Tension

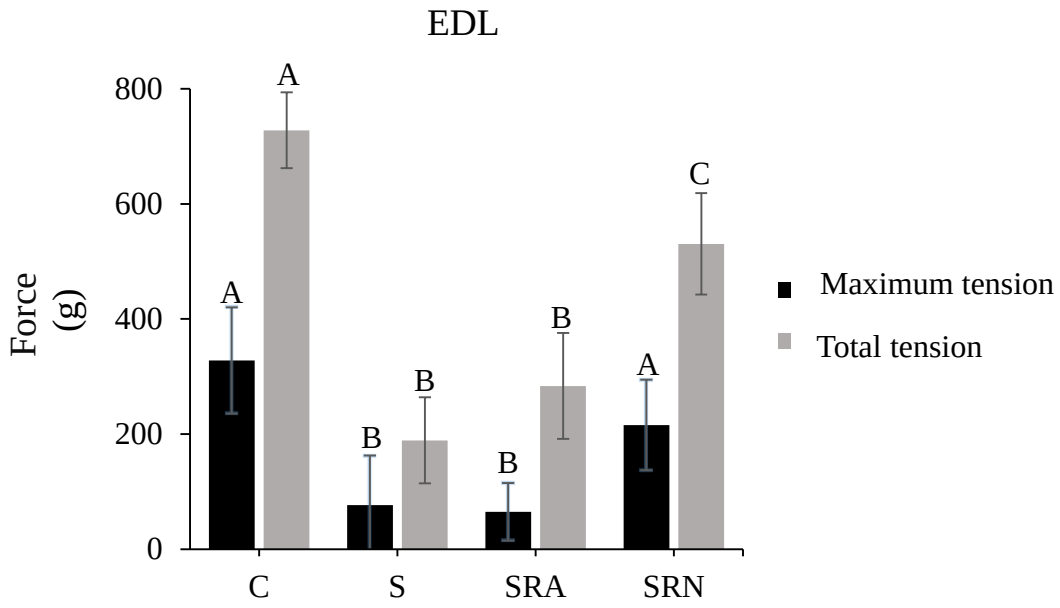


Fig. 7. Maximum tension and total tension of the EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

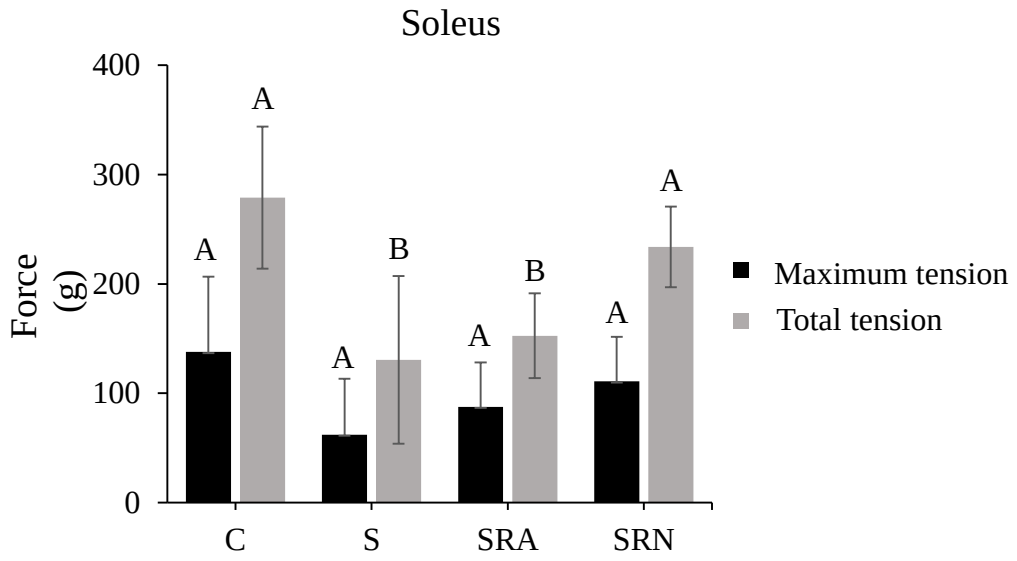


Fig. 8. Maximum tension and total tension of the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

Effect of the sweeteners sucrose, artificial *Stevia rebaudiana* and natural *Stevia rebaudiana* on the level of reactive oxygen species of skeletal muscle

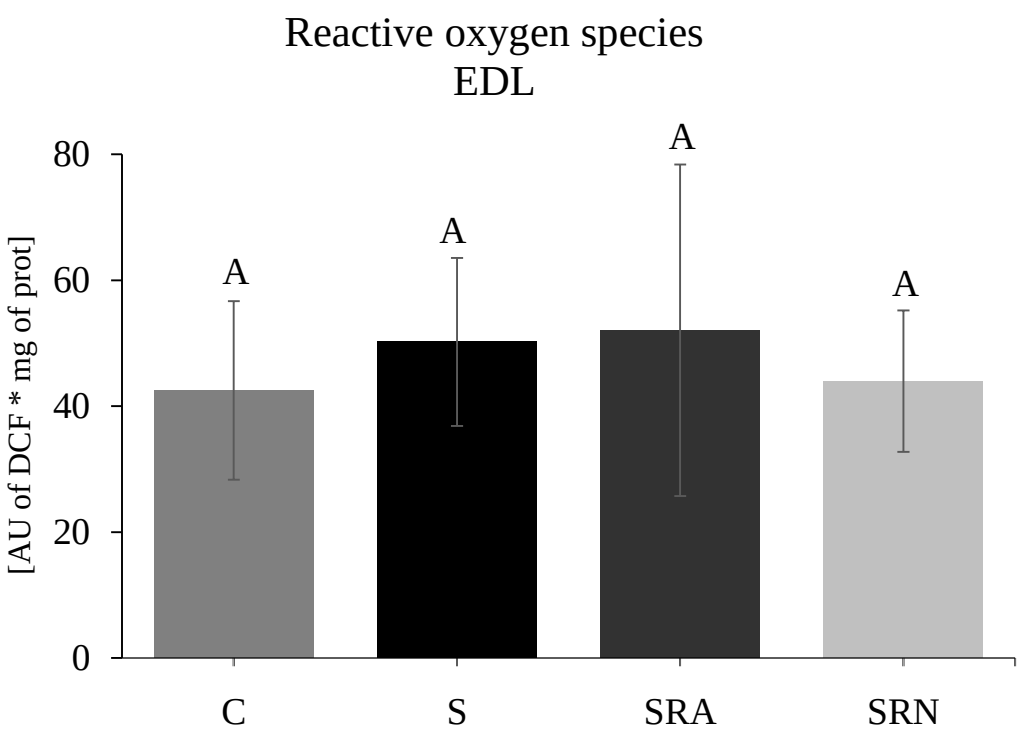


Fig. 9. Levels of reactive oxygen species in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

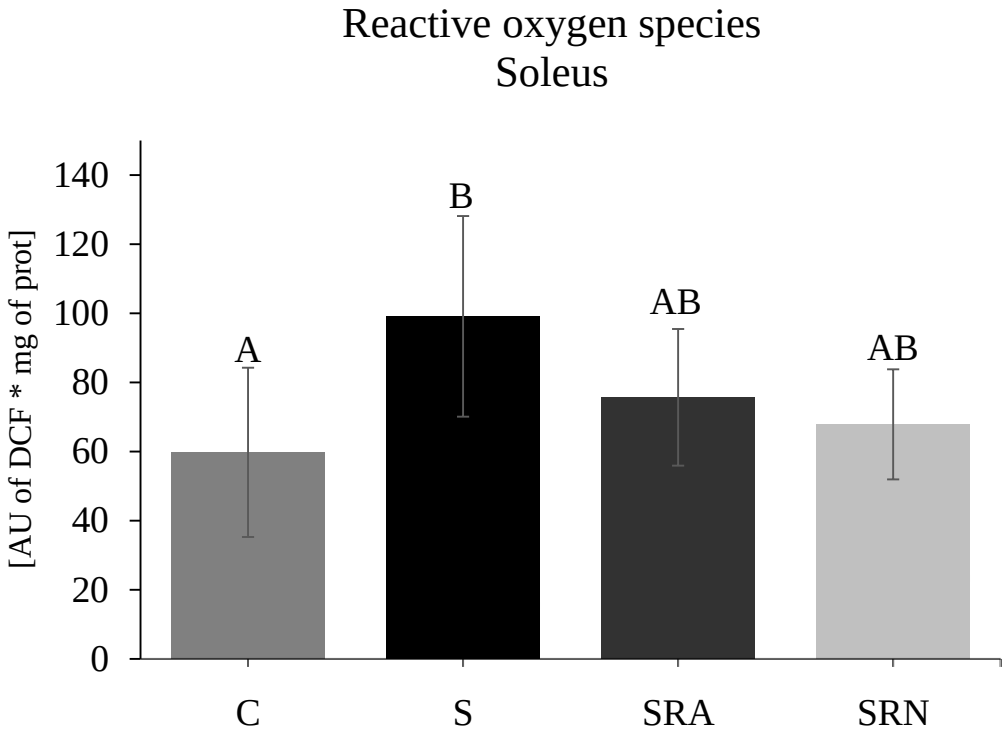


Fig. 10. Levels of reactive oxygen species in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

Effect of the sweeteners sucrose, artificial *Stevia rebaudiana* and natural *Stevia rebaudiana* on the antioxidant enzyme catalase (CA) of skeletal muscle

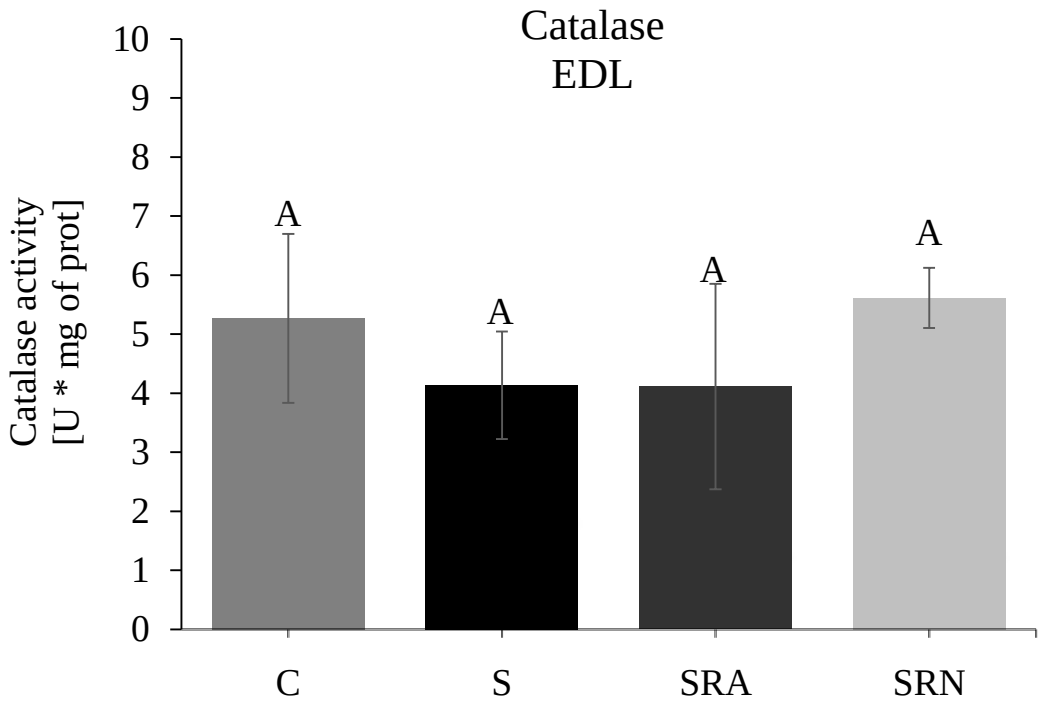


Fig. 11. Catalase levels in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

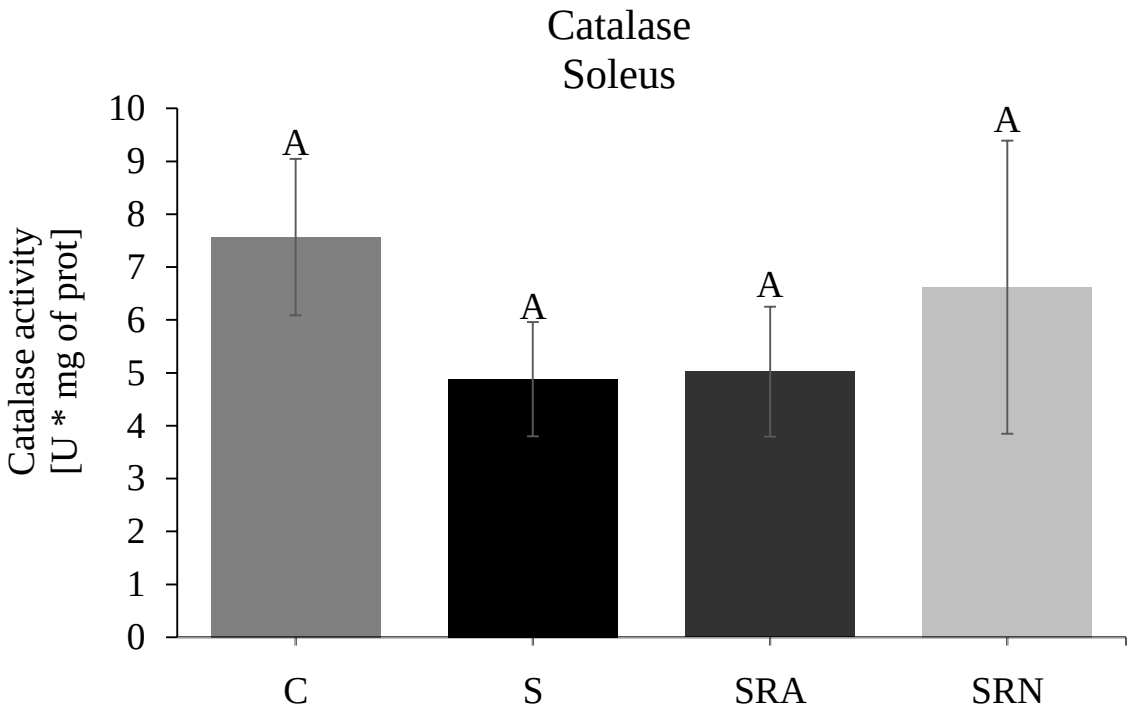


Fig. 12. Catalase levels in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions. C = control, S = Sucrose, SRA = Artificial Stevia rebaudiana, SRN = Natural Stevia rebaudiana; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey post-hoc test).

Conclusions

- The leaves of *Stevia* sp. Natural are an important alternative to conventional caloric sweeteners in combating the reduction of resistance time to muscular fatigue and on muscular strength.
- The glycosides extracted from Stevia sp. in synergy with artificial sweeteners they do not present the same effects.
- The antioxidant effects of Stevia sp. they only appear on deteriorated systems and not on healthy individuals.
- More studies are needed to obtain more conclusive results, because it appears that alternatives to sugar in the diet aimed at avoiding chronic diseases could increase the risk of these diseases.

Annexes

Perspectives

Future work should address the question of to what extent and under what circumstances the sweeteners in *Stevia rebaudiana* Bertoni, both natural and in synergy with artificial sweeteners used as a complementary treatment in metabolic diseases, modulate blood glucose levels, antioxidant defense, as well as its role on skeletal muscle fatigue in chronic diseases, particularly diabetes mellitus, to reveal the exact interference of Stevia sp. on the different routes deteriorated in pathological circumstances.

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