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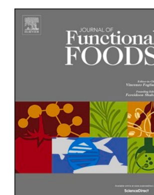

Sweet-inhibiting effects of gurmarin on intake during repeated acute and long-term sugar exposure: A behavioural analysis using an animal model

Raquel Rayo-Morales [a,b], Antonio Segura-Carretero [a], Nicolas Poirier [c], Loïc Briand [c], David Garcia-Burgos [b,*]

[a] *Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain*
[b] *Department of Psychobiology, Institute of Neurosciences, Centre for Biomedical Research, University of Granada, 18010 Granada, Spain*
[c] *Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne, F-21000 Dijon, France*

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ABSTRACT

Excessive consumption of added sugars is linked to chronic diseases, such as obesity, diabetes, dyslipidaemia or cardiovascular disease; and public health is searching for new strategies to reduce it. Although plant-derived bioactive compounds with inhibitory properties of sweet taste like *Gymnema sylvestre* show the potential to reduce sugar intake acutely, their impact after repeated administration is unknown. Therefore, we examined the changes of single and repeated exposures of a *Gymnema sylvestre* constituent, gurmarin, in sweet beverage consumption and preference in a preclinical model. 24 Wistar rats (50 % females) were divided into experimental (gurmarin) and two control groups (gymnemic acids or phosphate buffer solutions) according to the substances orally applied. Then acceptance and preference tests with sugar were performed within (Experiment 1) and between sessions (Experiment 2). We found that administering gurmarin decreased sucrose intake significantly, even after multiple treatments, without rebound effects. These findings suggest that sweet taste suppressors could be an effective tool for reducing long-term sugar consumption when repeatedly administrated.

1. Introduction

Our food environment is abundant in sweet, energy-dense products that are highly accessible and affordable, and often tailored to our innate taste preferences (Birch & Anzman-Frasca, 2011), which can lead to overconsumption (Drewnowski, 2004). In fact, sweeteners are added to 74 % of processed foods, including sauces, fruit juices, and meats (Popkin & Hawkes, 2016). More importantly, excessive sugar intake is a significant risk factor for health issues like obesity, diabetes, dyslipidaemia and cardiovascular disease (Johnson et al., 2007; Malik, Popkin, Bray, Després, & Hu, 2010), making reducing sugar consumption a public health priority (Jiao & Wang, 2018). Indeed, World Health Organization (2015) urges countries to take action in reducing sugar intake among both adults and children. For instance, adult intake in Europe varies from 7 to 8 % of total energy intake in countries like Hungary and Norway to 16–17 % in countries like Spain and the United Kingdom. Among children, the intake is even higher, with values ranging from about 12 % in countries like Denmark, Slovenia, and Sweden, to nearly 25 % in Portugal.

Besides their energy-carrying properties, the orosensory pleasure derived from the stimulation of sweet taste receptors is a crucial driver of reward-motivated eating independent of learning or prior experience, with significant implications for calories intake (Davis et al., 2007; de Araujo, 2011). To sweetness perception, the heterodimeric type 1 taste receptor 2 (T1R2)/type 1 taste receptor 3 (T1R3) plays a crucial role (Belloir, Neiers, & Briand, 2017). As a result, this receptor is being proposed as a key target for sweetness inhibition strategies. In particular, upon the idea of taste bud intervention (Rohde, Schamarek, & Blüher, 2020) through the implementation of sweet-taste-suppressing agents derived from natural plant compounds, the reduction of the pleasurable orosensory attributes is expected to make sugary products less appealing and decrease the desire to consume them (c.f. Kashima et al., 2020).

Examples of such plant-derived sweetness inhibitors include *Gymnema sylvestre* which contains gurmarin (a 35-residue polypeptide, active in rodents) and gymnemic acids (triterpenoid glycosides, active in humans), *Ziziphus jujube*, *Hovenia dulcis*, and lactisole (Fletcher, Pan, & Kinghorn, 2017; Sigoillot, Brockhoff, Meyerhof, & Briand, 2012). In

* Corresponding author at: Institute of Neurosciences, University of Granada, Avda. del Conocimiento s/n, 18100 - Armilla, Granada, Spain.
E-mail address: davidgb@ugr.es (D. Garcia-Burgos).

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humans, research has shown that rinsing the mouth with *Gymnema sylvestre* acutely decreases an individual's perceived taste liking of sweet stimuli (Brala & Hagen, 1983; Kashima et al., 2017, 2019). Using single applications, *Gymnema sylvestre* has also been found to reduce the total intake of snack-type foods (by 5.5 to 52.0 %) and sweet candies (Stice & Yokum, 2018; Stice, Yokum, & Gau, 2017) compared to individuals with normal perception. Additionally, a 23.0 % reduction in the quantity of chocolate or candy eaten compared to a placebo was observed in some studies (Nobel, Baker, & Loullis, 2017; Turner et al., 2020).

Contrary to the expected decrease in the intake of high-sugar sweet items, Turner et al. (2020) reported that some participants actually consumed more after using gymnemic acids. Interestingly, these same participants had given the high-sugar sweet food low pleasantness ratings. The authors attributed this phenomenon to a sense of curiosity, as the participants were initially unfamiliar with the sugar suppression effect of gymnema. Their desire to experience the effect led them to consume more; but the authors hypothesize that, with repeated exposure to the product, this curiosity factor would progressively diminish as participants become accustomed to the taste modulation. On the other hand, during early post-prandial periods, which is the period following a meal when sweet substance absorption is underway, Kashima et al. (2020) reported higher scores for the desire for sweet taste (0–20 min) after consuming *Gymnema sylvestre* compared to the control condition. Furthermore, Noel, Sugrue, and Dando (2017) found that a 1 % reduction in sweet taste response caused by *Gymnema sylvestre* was associated with a 0.40 g/L increase in the optimal concentration of sucrose. These studies suggest that diminishing sweet perception and hedonic response might acutely promote not only the desire for more intense stimuli to attain a satisfactory level of sweet reward but also influence eating habits, leading to increased sugar intake to compensate for a lower gustatory input (e.g., Kashima et al., 2020; Noel et al., 2017; Turner et al., 2020). Unfortunately, the impact of suppressing oral sweet sensations on long-term sugar consumption remains unexplored. In this sense, it is crucial to investigate not only the acute within-session effects but also the impact throughout the days of sweetness inhibitors in order to elucidate any potential downstream effects of the sweetness suppression strategy on energy intake and overall nutritional status.

To achieve this from a translational standpoint, an effective initial strategy is the preclinical animal approach under a highly controlled environment before proceeding to human trials. Thus, the main aim of this work was to examine the effects of a *Gymnema sylvestre* constituent, gurmarin, only applied on the tongue on short-term and long-term consumption and choice of sugar-sweetened beverages in an animal model. We predicted that, after applying gurmarin, inhibition of sweet taste, intake and preference ratio for these beverages would be reduced when compared with controls under repeated exposures within one session (Experiment 1) and under daily repeated exposures between sessions (Experiment 2). In animals, only one study has assessed the daily food intakes in rats fed diets containing powder of leaves of *Gymnema sylvestre* (with 3 % gurmarin at a concentration of 0.1 μg/ML). In this study, Katsukawa, Imoto, and Ninomiya (1999) reported that preference for sucrose decreased transiently by 13.2 % and 23.3 % respectively from the control levels at 1–2 days and subsequently recovered days later for a low concentration of sucrose (0.01 M), but not for higher concentrations (0.03 M and 0.1 M) or other no-sweet taste solutions. However, because *Gymnema sylvestre* was swallowed, it is difficult to separate the specific contribution of sweet taste receptor inhibition in oral and extraoral tissues on ingestive behaviour (Laffitte, Neiers, & Briand, 2014).

2. Materials and methods

The animals were kept in accordance with the guidelines set by EU Directive 2010/63/EU and Spanish Royal Decree 53/2013 for animal experiments. The experimental protocols of Experiments 1 and 2 were approved by the Research Ethics Committee at the University of Granada. All animal procedures carried out in this study were reviewed by animal-welfare officials and a designated veterinarian from the Animal Facility at the Biomedical Research Centre / University of Granada (UGR).

2.1. Experiment 1: Within-Session effects

Within-session effects refer to the immediate changes during the same session of the experiment. It involved measuring and comparing the sugar-related eating behaviour before and after the application of gurmarin to the tongue. The purpose was to gain insights into the acute effects of the gurmarin treatments on food intake and preferences, providing valuable information on the short-term impact of this intervention.

Subjects. A total of 24 experimentally Wistar rats (50 % females), weighing 267 g (male: 269 g and female: 264 g) at the start of the experiment, were used. The rats were housed individually in home cages and kept in a large colony room with a 12-hour light/12-hour dark schedule.

Sweet-inhibiting substances and sweet stimulus. To induce sweetness inhibition, we used recombinant Q1-gurmarin, a plant polypeptide that suppresses sweet taste, recombinantly expressed and purified as described in Sigoillot et al. (2012). The gurmarin was dissolved in 50 mM potassium phosphate buffer at a concentration of 30 μg/ML. This concentration (30 μg/ML) was selected based on previous studies in rodents (Murata et al., 2003; Ninomiya, Inoue, Imoto, & Nakashima, 1997; Ninomiya et al., 1998) and cellular-based assays (Sigoillot et al., 2018). Each rat received 20 μL of gurmarin solution on the dorsal surface of the anterior two-thirds of the tongue using a 10–100 μL micropipette (Eppendorf, Germany). In addition, two control groups received a lingual infusion with an equal volume at the same tongue location. Instead of gurmarin, one group received an herbal preparation of *Gymnema sylvestre* containing 75 % gymnemic acids (Arkure Health Care, Haryana, India) at 10 mg/ML concentration (Hellekant & Gopal, 1976), while the other group received a potassium phosphate buffer solution. Gymnemic acids were chosen as a control because they have been shown to be a bioactive compound ineffective in Wistar rats (Murata et al., 2003; Nakashima, Katsukawa, Sasamoto, & Ninomiya, 2001; Yamada et al., 2006) despite both compounds comes from the same *Gymnema sylvestre* plant. Also, gymnemic acids have an unpleasant sour taste that allowed us to further control for any confounding unpleasing tasting effects that might decrease sugar consumption. Given that traces of gurmarin might be found in the samples obtained from the herbal preparation of *Gymnema sylvestre* (Kamei, Takano, Miyasaka, Imoto, & Hara, 1992; Tiwari, Mishra, & Sangwan, 2014), we also estimated the concentration of gurmarin in this control group, which was $<$ 0.1 μg/ML. Regarding the sweet stimulus, we assessed the gurmarin-induced inhibitory sweet effects using sucrose solutions at 10 % (w/v) versus tap water, administered in 50 ML plastic tubes. New sucrose solutions were prepared daily at room temperature.

Behavioural tests. To measure intake and preference ratio, we used two different types of tests. One is a single-bottle acceptance test, in which the rats had access to 40 ML of a sucrose solution. Intake was measured in grams by weighing the tubes before and after each presentation at 5-minute intervals. The difference between the two weights indicated the amount of solution consumed (a value of 0.0 indicated that the animals did not drink anything). The other is a two-bottle preference choice test, in which the rats had 10 min of simultaneous access to 40 ML of water and 40 ML of sucrose solution. To calculate the preference ratio score for the second test, we used the following equation: sucrose solution intake / (sucrose solution intake + water intake). A score of 0.5 indicates equal intake of the sucrose solution and water, while a score of 1.0 indicates exclusive intake of the sucrose solution.

Procedure. The study involved conducting three phases: training, sweetness inhibition and sucrose exposure without suppressors during the light cycle in the rats' home cages. The animals were randomly

assigned to three weight-matched groups: Gurmarin, Gymnema and Buffer ($n = 8$ rats / group, 50 % females). During the 1–3 day training phase, the rats were handled for 5 min per day, food was removed from their home cages to help them adapt to the food deprivation schedule for three days before the sweetness inhibition sessions and we established a baseline for water consumption in order to control for any initial differences in fluid intake. Throughout the experiment, the rats were provided with 90-minute access to the standard diet in their home cages in the afternoon. The sweetness inhibition sessions took place on days 4 and 5. Prior to each testing session, water was removed from the cages two hours beforehand. The rats were anaesthetized using isoflurane (5 % for induction and 1–2 % for maintenance), and the corresponding substances were applied to the tongue according to their group at 10:00 a.m. After a 3-minute interval, the corresponding test was performed. The single-bottle acceptance test was conducted on day 4 in which the sucrose solution was presented at 5, 10, and 15 min. The next day, the two-bottle choice test (sucrose versus water) was first carried out. Second, in order to examine the potential post-prandial rebound effect after using bioactive compounds with sweet-taste-suppressing activity, another single-bottle acceptance without any lingual application of substances was also conducted 120 min later in which the sucrose solution was presented at 5 and 10 min.

2.2. *Experiment 2: Between-Session effects*

Between-session effects refer to the changes in sugar-related eating behaviour observed across different time points in the experiment, in which the same treatment was administered in separate days. The aim was to assess the cumulative influence of the gurmarin treatment with repeated exposure to specific solutions containing sucrose across multiple sessions, and its impact on the rats' consumption and preference for those solutions.

Subject and methods. Following the principles of the 3Rs, we used the same 24 animals as in Experiment 1. We distributed them orthogonally across the 3 groups of 8 animals in each group to control the impact of any previous experience. The housing conditions, sweet-taste-suppressing substance application and preparation methods, training phase, water deprivation schedules, baseline for water consumption, and behavioural tests were consistent with those used in Experiment 1.

Sweet Stimuli. Sugar-sweetened beverages were prepared using tap water and consisted of 1 % (v/v) vanilla or 0.7 % (v/v) lime extracts (Dr. Oetker, Germany) and 10 % sucrose (w/v) compound. These beverages were presented to the rats in 50 ML plastic tubes. The Flavour A contained vanilla for half of the rats, and lime for the other half. The Flavour B was the reverse of the Flavour A for each group. Flavours were counterbalanced across all rats.

Procedure. The experiment was conducted in three phases: training, exposure and sweetness inhibition. After the training phase as in Experiment 1 (days 1–3), the exposure phase lasted for 8 days (days 5–8 and days 10–13), during which rats were given 10-minute access to 40 ML of the Flavour A with sucrose at 11:00 a.m. and the Flavour B with sucrose at 1:00 p.m., with the flavour presentations reversed daily, only under water deprivation. The sweetness inhibition phase was carried out using the Flavour A with sucrose over four days (days 16–19) under water and food deprivation to match the conditions of Experiment 1. Additionally, two-bottle preference tests were conducted before, during, and after exposure (days 4, 9, and 14) under water deprivation as well as before and after sweetness inhibition (days 15 and 20) under water and food deprivation. These tests compared the intake (in grams) of Flavour A alone (without sucrose) versus water at 11:00 a.m. and the Flavour B alone (without sucrose) versus water at 1:00 p.m. to determine whether the rats displayed a hedonic shift or preference bias towards one of the two conditioned flavours associated with sucrose throughout the experiment. To calculate the preference ratio score, we used the following equation: Flavour A or B intake / (Flavour A or B intake + water intake).

3. Data analysis

For each rat, we measured their intake of water or sugar-sweetened beverages in grams, as well as their preference for sugar-sweetened beverages or flavours as a ratio. We then calculated the mean values and standard error of the mean (SEM) for the data. In Experiment 2, we further analysed changes in sugar intake levels following lingual treatment by subtracting the pre-treatment intake from the post-treatment intake for each day, with sugar solution intake measured in grams. To analyse the data, we used an analysis of variance (ANOVA) for normally distributed data, with group (Gurmarin vs. Gymnema vs. Buffer) and sex (male vs. female) as between-subject factors, and time (defined as minutes in Experiment 1 and days in Experiment 2) as within-subject factors. Additionally, we analysed the consumption of flavour A during sweetness inhibition using a repeated-measures of covariance (ANCOVA) with intake of that specific flavour phase during the last day of the exposure phase as a covariate. When deviations from the assumption of sphericity were found, Greenhouse-Geisser correction was applied. The effect size was estimated using eta partial square ($\eta p2$). Reliable interactions were followed, when appropriate, by simple effects analyses. To identify differences among groups, post-hoc Tukey contrasts were performed or pairwise comparison adjusted by the Bonferroni. If the sample did not have a normal distribution, the results were analysed using the Kruskal-Wallis test. All calculations were performed using the statistical software package IBM SPSS Statistics 28.0.1.0. The level of significance was set at $p \leq 0.05$, with the abbreviation "ps" used for multiple p-values.

4. Results

4.1. *Experiment 1: Within-Session effects*

Baseline for water consumption. The mean intake of water values in baseline at 5 min were 1.56 ± 0.46 for the Gurmarin group, 2.04 ± 0.46 for the Gymnema group and 1.48 ± 0.33 for the Buffer group. At 10 min, the mean was 2.04 ± 0.45 for the Gurmarin group, 2.33 ± 0.49 for the Gymnema group and 2.16 ± 0.36 for the Buffer group. Finally, at 15 min, the mean was 3.46 ± 0.79 for the Gurmarin group, 2.85 ± 0.52 for the Gymnema group and 3.31 ± 0.49 for the Buffer group. No differences were found among the groups in any of the baseline data, including at 5 min ($F(1.355, 20.326) = 1.829$, $p = .192$), 10 min ($F(1.355, 20.326) = 1.357$, $p = .270$), or 15 min ($F(1.355, 20.326) = 0.541$, $p = .530$).

Intake of sugar solution during sweetness inhibition. The 2 (Sex) $\times$ 3 (Group) $\times$ 3 (Time: 5 min vs. 10 min vs. 15 min) ANOVA revealed significant main effects of group ($F(2,28) = 4.065$, $p < .05$, $\eta p2 = 0.31$), and time ($F(1.453,26.156) = 515.505$, $p < .001$, $\eta p2 = 0.99$). No other main effects or interactions were significant (largest $F(1.453,26.156) = 3.067$, $p = .78$). Data of the acceptance test at three intervals (5, 10, and 15 min) among three groups are shown in Fig. 1. On the one hand, a decreased consumption of the sugar-sweetened solution was observed in the Gurmarin group compared to the Buffer group ($p < .05$). Specifically, the mean intake of sucrose in the Buffer group (100 %) significantly reduced to 38.46 %, 20.51 %, and 7.76 % at 5, 10, and 15 min, respectively, in the Gurmarin group. On the other hand, an increase in consumption over time, with higher consumption at 15 min compared to 10 min, and both compared to 5 min ($ps < .05$).

Preference for sugar beverages under sweetness inhibition. The mean preference values for sugar solutions were 0.92 ± 0.02 for the Gurmarin group, 0.94 ± 0.01 for the Gymnema group, and 0.88 ± 0.04 for the Buffer group. In the case of sex, the mean values were 0.89 ± 0.03 for males and 0.94 ± 0.01 for females. Accordingly, no significant effects of group ($F(2) = 2.48$, $p = .30$) or sex ($F(1) = 1.49$, $p = .22$) were observed.

Intake of sugar solution post-sweetness inhibition. The 2 (Sex) $\times$ 3 (Group) $\times$ 2 (Time: 5 min vs. 10 min) ANOVA revealed a significant effect of time ($F(1,18) = 274.79$, $p < .001$, $\eta p2 = 0.93$) and a marginal

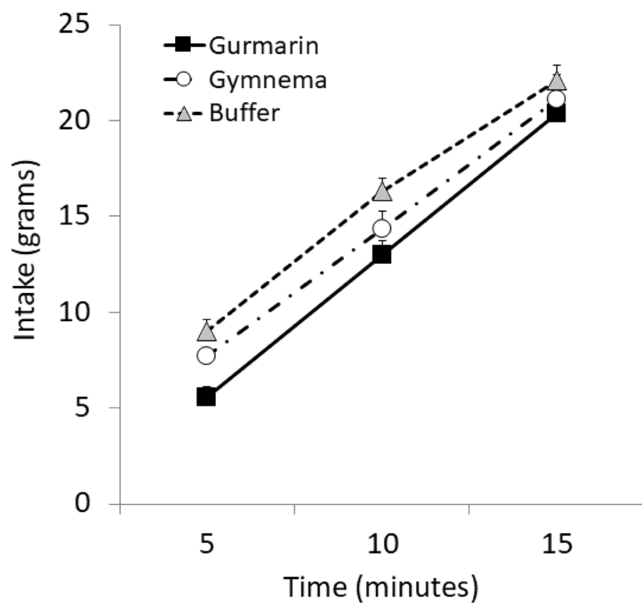

Fig. 1. Effect of sweet taste inhibitor Gurmarin on sugar intake compared to control groups (Gymnema and Buffer) at three-time intervals (5, 10, and 15 min) under food deprivation conditions. The data presented as mean (±SEM) was collapsed across sex since there was no significant effect.

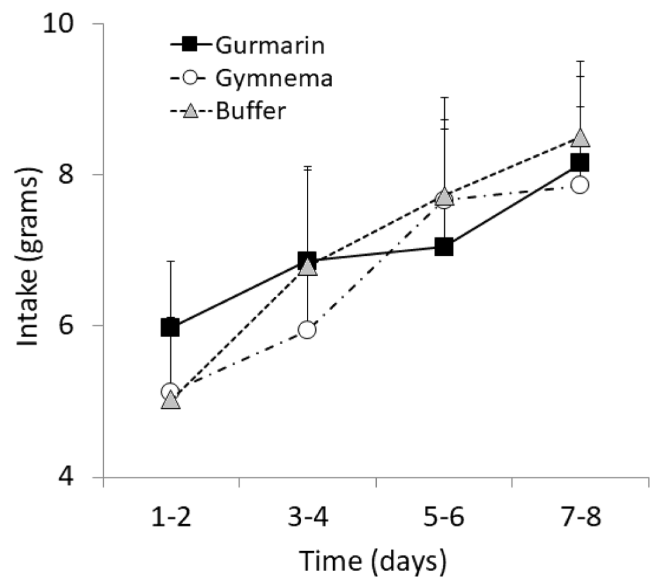

Fig. 2. The intake of sugar-sweetened beverages during the exposure phase (grouped into 2-day blocks) for the experimental group (Gurmarin) and two control groups (Gymnema and Buffer) without food deprivation. The data presented as mean (±SEM) was collapsed across flavour and sex, as there was no significant effect observed in these factors.

effect of group ($F(1,18) = 3.36$, $p = .06$, $\eta p2 = 0.72$). No other main effects or interactions reached significance (largest $F(1,18) = 2.73$, $p = .11$). Specifically, the consumption of the sugar solution increase from 5 min (1.7 ± 0.2) to 10 min (2.9 ± 0.2) ($p < .001$). On the other hand, there was no evidence of a rebound effect in the Gurmarin group (3.20 ± 0.54), which did not differ from the Buffer control group (3.43 ± 0.36). In comparison, the Gymnema control group showed a trend towards lower consumption (2.08 ± 0.20) ($p = .83$).

4.2. Experiment 2: Between-Session effects

Intake of sugar-sweetened beverages. During the exposure phase, the 2 (Sex) × 3 (Group) × 2 (Flavour) × 8 (Time: day 1 vs. day 2 vs. day 3 vs. day 4 vs. day 5 vs. day 6 vs. day 7 vs. day 8) ANOVA only showed a significant effect of time ($F(4{,}25, 76{,}44) = 21.12$, $p < .001$; $\eta p2 = 0.54$) and sex ($F(1, 18) = 5.67$, $p < .05$; $\eta p2 = 0.24$). No other main effects or interactions were significant (largest $F(4{,}25, 76{,}44) = 1.62$, $p = .18$). On the one hand, the data shows an increase in sugary beverage consumption between day 1 and days 3, 4, 7 and 8 as well as between days 2 and 6 and day 8 of exposure (ps < .05). To provide a clearer representation of this trend, sugar-sweetened beverage consumption data during the exposure phase was grouped into 2-day blocks in Fig. 2. Additionally, the study found that female rats (average of 7.68 ± 0.17 g) consumed more sugar-sweetened beverages than male rats (average of 6.10 ± 0.16 g).

During the sweetness inhibition phase, a 2 (Sex) × 3 (Group) × 4 (Time: day 1 vs. day 2 vs. day 3 vs. day 4) ANCOVA and ANOVA were performed on intake and change in intake, respectively. When we examined the intake of flavour A with sucrose, we found a significant main effect of time ($F(2.20, 44.06) = 2.89$, $p < .001$; $\eta p2 = 0.31$) and a marginal effect of group ($F(2, 20) = 2.576$, $p = .07$; $\eta p2 = 0.22$). No other main effects or interactions were significant (largest $F(4.55, 38.71) = 1.45$; $p = .23$). Specifically, the Gurmarin group consumed less flavour A with sucrose compared to the buffer group ($p = .051$; see Fig. 3A). Additionally, we observed a significant decrease in consumption on day 1 compared to days 3 and 4, as well as on days 2 and 3 compared to day 4 (ps < .05). Concerning the change in intake (Fig. 3B), the analysis yielded significant main effects of sex ($F(1,18) = 5.024$, $p < .05$; $\eta p2 = 0.22$), group ($F(2,18) = 4.58$, $p < .05$, $\eta p2 = 0.38$) and time ($F(3,54) = 29.174$, $p < .001$; $\eta p2 = 0.62$). No significant interactions were observed (largest $F(6,54) = 1.309$, $p = .27$). Although both sexes indicated an upward change in the consumption of flavour A with sucrose during the sweetness inhibition phase, female rats showed a lower increase (1.47 ± 0.29) compared to male rats (4.69 ± 0.29). On the other hand, the group Gurmarin did not increase their consumption of flavour A during sweetness inhibition, and showed a minimal change in intake, averaging 0.004 g/day. In contrast, both control groups demonstrated a significant upward change in consumption compared to Gurmarin group (ps < .05), with an average increase of over 4 g per day during this phase. This resulted in an average percentual increase of 51.87 % in the Gymnema and 71.20 % in the Buffer groups. No significant differences were found between both control groups. Finally, a positive change value across days was also detected with day 1 < days 2, 3 and 4 as well as days 2 and 3 < day 4 (ps < .01).

Preference for flavours previously paired with sugar. The preference for flavour A was analysed taking into account the exposure phase and the sweetness inhibition phase. In the first case, a 2 (Sex) × 3 (Group) × 2 (Flavour) × 3 (Test: before, during, and after exposure phase) ANOVA revealed a significant main effect of test ($F(2,36) = 10.85$, $p < .01$, $\eta p2 = 0.37$), indicating that animals' flavour preferences changed over time. Specifically, animals showed a lower preference for flavours before (0.56 ± 0.03) and after (0.55 ± 0.03) the conditioning phase compared to during the conditioning phase (0.67 ± 0.03) (ps < .01). No other main effects or interactions were significant (largest $F(2,18) = 2.86$, $p = .084$).

In the case of sweetness inhibition, a 2 (Sex) × 3 (Group) × 2 (Flavour) × 2 (Test: before and after sweetness inhibition) ANOVA showed a significant effect of test ($F(1,18) = 35.60$, $p < .001$, $\eta p2 = 0.64$) and significant interactions of Group × Sex × Test ($F(2,18) = 6.10$, $p < .01$, $\eta p2 = 0.40$) and Group × Sex × Flavour × Test ($F(2,18) = 6.94$, $p < .01$, $\eta p2 = 0.43$). The analysis of simple effects indicated that male rats in the Gurmarin and Buffer groups increased their preference for flavour A between the pre-sweetness inhibition test (0.66 ± 0.07 and 0.73 ± 0.06) and the post-sweetness inhibition test (0.90 ± 0.04 and 0.84 ± 0.04, respectively). Moreover, female rats showed a higher preference for flavour A during the test after sweetness inhibition (0.75 ± 0.07) than male rats (0.65 ± 0.07) ($p < .05$).

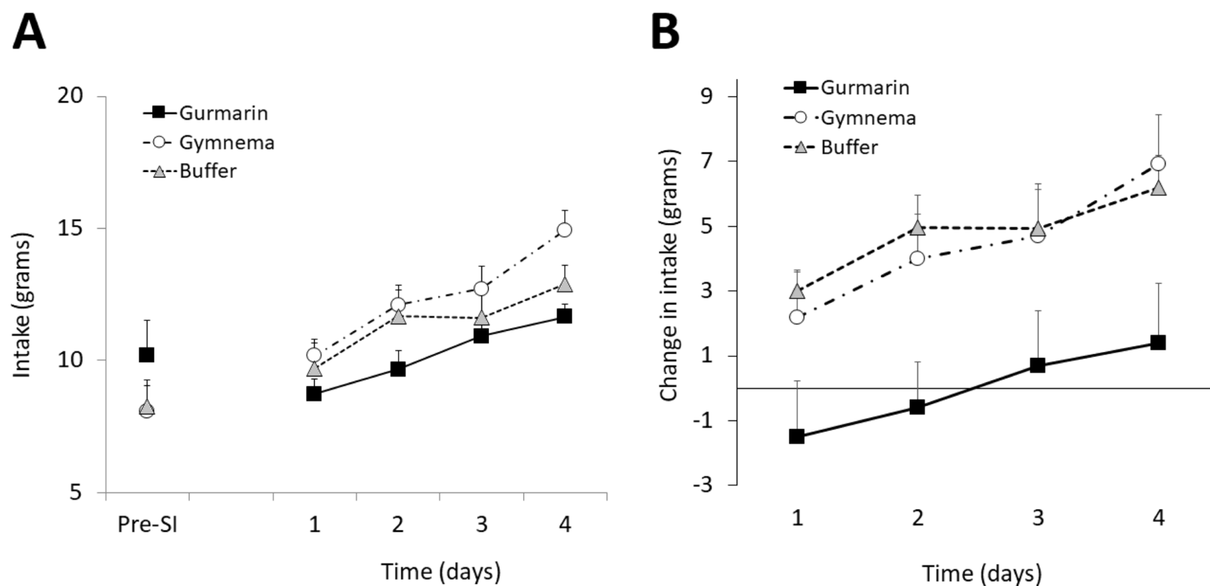

Fig. 3. Response to Flavour A with sucrose during a 4-day sweetness inhibition phase with food deprivation among three groups: the experimental group (Gurmarin) and two control groups (Gymnema and Buffer). A) Average intake of Flavour A with sucrose before (pre-sweetness inhibition; Pre-SI) and after lingual administration. B) Average change in intake of Flavour A with sucrose during the sweetness inhibition phase. The data, presented as mean (±SEM), has been collapsed across sex as no significant effect was observed.

5. Discussion

The main objective of this preclinical study was to examine the impact of gurmarin, a derivative of *Gymnema sylvestre*, applied only on the tongue, on short-term and long-term consumption and choice of sugar-sweetened beverages in rats. Our first hypothesis was that inhibiting sweet taste with gurmarin should decrease the intake and preference ratio for these beverages in comparison to control groups during repeated exposures within one session (Experiment 1). Our analysis of the consumption data supported our hypothesis, as the Gurmarin group exhibited decreased sugar intake compared to the Buffer control group. By contrast, no such difference was observed in the second control group, Gymnema. The intermediate sugar consumption in this group may be attributed to several factors, including the potential unpleasant taste of gymnemic acids and/or the presence of residual gurmarin in the applied extract. Although some studies have demonstrated an inhibitory effect of gurmarin even at low concentrations such as 3 μg/ML (e.g., Ninomiya, Inoue, & Imoto, 1998), the relatively low estimated gurmarin concentration in this control group (<0.1 μg/ML) makes it difficult to explain the results solely based on gurmarin's presence. Our estimation of gurmarin content was derived from previous experimental characterization of dry *Gymnema sylvestre* leaves, which has been reported to contain as low as 10 p.p.m. of gurmarin (e.g., Imoto, Miyasaka, Ishima, & Akasaka, 1991; Katsukawa et al., 1999). However, it should be mentioned that obtaining gurmarin from its natural source poses challenges, which usually requires chemical synthesis and refolding into an active form (Sigoillot et al., 2012). Moreover, the herbal extract of *Gymnema sylvestre* utilized for our control group primarily targeted gymnemic acids, not gurmarin. Consequently, it is plausible that the actual gurmarin content in our herbal extract for the control group may be even lower. In any case, the specific effects of the residual gurmarin content and the potential influence of the unpleasant taste on sugar consumption remain to be experimentally determined.

Our second hypothesis proposed that the consumption and preference for sugar-sweetened beverages should decrease during repeated gurmarin applications among daily sessions. Our intake data supported this hypothesis, with the Gurmarin group consuming less flavour A with sucrose than the Buffer group after 4 days of testing. The results showed that the effectiveness of gurmarin in acutely reducing sugar intake was similar to a previous behavioural study in C57BL mice. This earlier study used a short-term lick test to measure the number of licks for sweet substances with a similar concentration of sugar (0.5 M [17 % weight/volume]) and 3 mM quinine, as well as oral infusions of 30 μg/ML gurmarin. They found that the number of licks decreased to 55 % of the control level when the rats were given the sugar mixture for the first time after 10 or 20 min of treatment with gurmarin (Murata et al., 2003).

However, despite our initial predictions that gurmarin would reduce animals' preference for sugar solutions (Experiment 1) or flavours associated with sugar (Experiment 2), no significant differences were observed between the Gurmarin and control groups. Interestingly, in both experiments, animals showed a preference rate for sugar-related beverages of over 80 % after gurmarin administration. This suggests that, despite being exposed to a sweet-taste-suppressing treatment, the animals' choice for sugar-related items was strong enough to outweigh the alternative option of water. Based on the traditional understanding that acceptance records consummatory behaviour while preference measures the motivation for that behaviour (e.g., Burrows, 1952), it can be argued that gurmarin was able to interfere with taste-elicited sugar intake responses but not the drive to seek sugar in our case. Nevertheless, more research on the behavioural taste response with specific paradigms for measuring consummatory behaviour (e.g., intake), sweet taste perception (e.g., lickometer), taste hedonics (e.g., using orofacial relativity test) and motivation (e.g., conditioned approach or Pavlovian-instrumental transfer test) is needed to confirm such possibility (Berridge & Robinson, 2003; Gaillard & Stratford, 2016).

Regarding the mechanisms of how gurmarin suppresses sugar intake, animal research has revealed that gurmarin's receptor-interacting site is located on the apical side of taste cells, achieved through selective synaptic coupling with gurmarin-sensitive receptors. As a result, gurmarin selectively inhibits only a subset of sucrose-best chorda tympani fibers. Subsequently, the central nervous system's nucleus of the solitary tract integrates information from both gurmarin-sensitive and insensitive receptors, along with other taste nerves, leading to the corresponding reduction of the taste-elicited consummatory behavioural response when chorda tympani is inhibited (Lemon, Imoto, & Smith, 2003). Consequently, there is a decreased acceptance of sugar. However, contrary to its impact on local reflex pathways, gurmarin might have a

relatively smaller influence on motivational functions as shown by the involvement of mesolimbic dopaminergic neurons in driving preferences for sucrose during two-bottle preference tests designed to gauge the 'reward value' of sugar. Moreover, the role of mesolimbic dopaminergic neurons becomes prominent in determining the reward value of sugars beyond their sweet taste. Thus, burst firing of mesolimbic ventral tegmental area dopaminergic neurons have been found to be crucial for the reinforcing effects of intra-gastric sucrose in a food-seeking task (Liu & Bohórquez, 2022). In such case, the high preference for sucrose might potentially arise from its motivational post-absorptive outcomes, and relatively independent of its sweet taste suppression.

Focused on the potential rebound effect, heightened preference and/or increased hedonic response to sweet foods caused by sweetness blockers has been a topic of debate in the human literature, as seen in Kashima et al. (2020), Noel et al. (2017), and Turner et al. (2020). There is concern that individuals may overcompensate and consume more of a pleasant substance after its consumption has been restricted, which could limit the clinical applications of sweetness blockers. In our study, we found no significant differences in repeated sugar consumption between the Gurmarin and Buffer control groups when rats were reintroduced to sugar alone. However, caution must be taken when comparing our results with other human studies, such as Noel et al. (2017), which used repeated administrations of *Gymnema sylvestre* with sucrose concentrations over four days, due to potential genetic differences in sweet taste receptors between Wistar rats and humans. For example, while gymnemic acids have no inhibitory effect on taste in mice and rats, they affect the sweet taste in humans, and gurmarin inhibits sweet perception in rats but not in humans (Sigoillot et al. (2012)).

Other factors that may affect the effectiveness of sweetness blockers include differences in physiology, metabolism, doses, and the complexity of human biology or clinical settings. Moreover, the duration of the initial post-treatment reduction, the duration of the treatment and previous experience with sugar are also relevant parameters that must be considered. For instance, given that the effect of gurmarin is not immediate and requires approximately 5 min to achieve maximal suppression of sweet responses recorded from single mouse fibers (Ninomiya, Imoto, & Sugimura, 1999), it would be valuable to explore alternative testing temporal parameters. Thus, conducting the test after a 5-minute post-treatment interval, instead of the current 3-minute interval used in this study, might further decrease acceptance and preference for sugar.

Methodologically, it is important to note another limitation related to the duration of the two-bottle test. The use of a 10-minute test may have included both gustatory and post-ingestive cues that can modulate fluid intake behaviour. To minimize the contribution of post-ingestive cues, a better approach would have been to use short-term two-bottle tests (<5 min) that account for minute-to-minute fluctuations in taste sensitivity (Gaillard & Stratford, 2016). Moreover, the inclusion of sweetened solutions that are relatively inert in the gut or that possess varying levels of calories may also be helpful in future studies, such as saccharin and sucralose acesulfame potassium considered "non-nutritive sweeteners" (few or no calories), or aspartame as a "nutritive sweetener" (adds some calories but far less than sugar). This approach will allow to effectively investigate the influence of calorie levels on sweet-taste suppressors.

In summary, the present study pointed out that administering sweet-taste suppressors like gurmarin resulted in a significant decrease in sucrose intake within and between sessions, and there was no rebound effect in sugar consumption when reintroducing sugar after administering sweet-inhibiting treatments. Accordingly, suppressors might have the potential to be an effective tool for reducing sugar consumption when repeatedly applied. However, the animals' choice of sugar-related items was strong enough to outweigh the alternative option of water, indicating that gurmarin was able to interfere with sugar intake but not the desire to consume sugar. Further investigation into the behavioural taste response is necessary, utilizing specific paradigms that can distinguish between the consummatory and preparatory aspects of sugar intake. Such research is crucial in establishing the underlying mechanisms involved in preventing or reducing long-term consumption of food, which can have a significant impact on an individual's body weight status.

6. Ethics statement

The animals were kept in accordance with the guidelines set by EU Directive 2010/63/EU and Spanish Royal Decree 53/2013 for animal experiments. The experimental protocols of Experiments 1 and 2 were approved by the Research Ethics Committee at the University of Granada. All animal procedures carried out in this study were reviewed by animal-welfare officials and a designated veterinarian from the Animal Facility at the Biomedical Research Centre / University of Granada (UGR).

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CRediT authorship contribution statement

Raquel Rayo-Morales: . **Antonio Segura-Carretero:** Writing – review & editing, Funding acquisition. **Nicolas Poirier:** Methodology. **Loïc Briand:** Writing – review & editing, Resources, Funding acquisition. **David Garcia-Burgos:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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