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Suppression of sweet taste-related responses by plant-derived bioactive compounds and eating. Part II: A systematic review in animals

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ABSTRACT

This article, the second in a two-part series, continues the discussion on the nature of the relationship between the level of sweet taste suppression and eating behaviour, but in animal rather human subjects. In particular, the aim was to review the scientific literature on the impact that bioactive compounds that decrease oral sweet sensations have on intake, preference and physiological status in preclinical studies. This review was registered in the *International Prospective Register of Systematic Reviews* and conducted according to the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) and the *Scottish Intercollegiate Guidelines Network* and covered original papers included in Web of Science, PubMed, Scopus, Food Science Source and Food Science and technology abstracts. We identified 28 peer-reviewed English-language studies that fit the topic and met the inclusion criteria. We identified three plant species, *Gymnema sylvestre*, *Hovenia dulcis*, and *Ziziphus jujuba*, that possess acute sweetness-inhibitory properties. When administered orally, these plants reduced neural responses to sweet stimuli and decreased consumption. However, studies on the longer-term effects of antisweet activity remain to be conducted. Translating the valuable insights into the mechanisms underlying the relationship between sweet taste impairment and eating behaviour into practical clinical applications are discussed.

1. Introduction

The current global obesity epidemic is a result of various dietary factors, including the overconsumption of sugars. Indeed, extensive research has consistently demonstrated a positive correlation between added sugar intake and the risk of developing obesity and related health conditions [1]. Likewise, a meta-analysis of 30 randomized controlled trials found that reducing intake of added sugars led to significant reductions in body weight and improvements in metabolic health markers, such as blood pressure and blood lipids [2]. These results underscore the importance of strategies that help reduce consumption of added sugars for prevention and treatment of these health problems.

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Interventions that temporarily block sweet taste receptors, such as those performed using plant-derived bioactive compounds with antisweet activity, may provide an additional strategy to address obesity and eating disorders [3], in particular when considering the scarcity of effective interventions currently accessible [4–6]. However, the precise mechanisms underlying the inhibition of sweetness to regulate eating, as well as the mechanisms of these bioactive compounds, remain to be clarified. In this context, animal studies may be critical in advancing our understanding. It is important to note that mammalian species such as mice, hamsters, rats and primates share similar sweeteners preferences with humans [7] and that tasting something sweet is also known to evoke a strong hedonic response in these animals. As in humans, this preference for sweetness is thought to have evolved as a survival mechanism, helping animals identify and seek out high-energy food sources [8].

Therefore, our purpose was to review the scientific literature on the impact that plant-derived bioactive compounds that decrease oral sweet sensations have on intake, preference and physiological status in preclinical studies. The secondary aim was to examine the effects of bioactive compounds in inhibiting sweet taste in populations with unhealthy status associated with obesity and abnormal eating behaviours to gather preliminary information regarding dosages, conditions and effectiveness of such interventions. In doing so, our objective was two-fold. Firstly, to identify suitable animal models to investigate sweet inhibition and determine optimal study methodologies that can be translated into clinical applications to effectively control eating behaviour. Secondly, to elucidate the mechanisms underlying sweetness inhibition of plant-derived bioactive compounds, spanning from molecular interactions to the modulation of eating behaviour.

2. Materials and methods

2.1. Protocol and registration

This systematic review was performed following the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) [9]. It was registered in the *International Prospective Register of Systematic Reviews* (PROSPERO, CRD42021248971, May 15, 2021) for both humans and animals.

2.2. Inclusion and exclusion criteria

The inclusion criteria to select the articles were: 1) publications on compounds with anti-sweet taste capacity; 2) animal studies; 3) oral application; 4) the effects in behavioural, neural and/or metabolic terms; 5) original research articles, including controlled trials, cohort study, case-control study, cross-sectional study, crossover study, case report, case series reports and animal research studies; and 6) publications in English. All published papers were searched regardless of the date of publication.

Studies were excluded if they were: 1) not based on original quantitative data such as reviews, commentaries, meta-analyses, book chapters, opinion reports, guidelines or editorial articles; 2) pharmaceutical medications; 3) substances with a synthetic origin; 4) focused only on the purification, structures, cellular activity, molecular biology or food development of substances with anti-sweetness activity; 5) based only on other-than-sweet-taste suppressions (such as bitter taste inhibition); 6) forms without available full-texts; and 7) duplicate papers.

2.3. Search and selection of studies

The search was implemented from May 2021 to January 2022 in six databases: Web of Science, PubMed, Scopus, Food Science Source (FSS) and Food Science and technology abstracts (FSTA). The terms used were: “bioactive”, “plant”, “herbal”, “sugar”, sweet*, inhibit*, suppress*, block* and “taste”. They were applied in all databases, and Boolean operators “AND” and “OR” were used.

The process of removing duplicate citations and screening was facilitated using the Rayyan Platform (https://rayyan.qcri.org; [10]). Two independent reviewers conducted the final screening and assessed eligibility of the articles. Discrepancies were rechecked until a consensus was reached. Finally, a citation analysis was carried out to find additional studies meeting the criteria, which were classified as “identified from other sources”.

2.4. Data extraction

A data list was extracted into a Microsoft Excel (2016) worksheet to record the following items for each eligible study: characteristics of the subjects (age, gender), characteristics of the bioactive compounds (studied plant, plant of origin, commercial product name, preparation, administration route, concentration and time of administration), outcomes of interest, and standard information about the study (participant, study type, measure, control stimuli, evidence level or days of treatment).

2.5. Outcomes

The primary outcomes of interest of this systematic review were changes related to sweet stimuli after application of sweet-taste blockers in: (1) taste perception during oral antisweet stimulation via neural and behavioural techniques (temporal, intensity and hedonic characteristics of sweetness); (2) eating behaviour (consumption and preference for sweetened items) with post-oral antisweet actions (appetite/desire to eat/motivation and intake/consumption); and (3) physiological status following sweet-taste blockers (saliva). Additionally, we examined secondary outcomes related to taste perception, eating behaviour and physiological status in non-

healthy populations.

2.6. Statistics

Descriptive analyses were used to present the results, and the most significant data were summarized in tables. “0″ represents no statistically significant difference ($p > .05$), while "+" represents a significant difference ($p \leq .05$) when comparing the bioactive compound and the control conditions.

3. Results

3.1. Study selection

The systematic literature search identified 899 eligible studies. After manual and automatic deduplication, 344 studies were excluded. Then, through screening for title and abstract, 531 studies were eliminated. The full text of the remaining 24 publications was reviewed following inclusion and exclusion criteria and quality assessment. After screening full-text manuscripts, a total of 23 papers were eligible. 5 additional articles were retrieved through reference screening of selected papers, making a total of 28 articles. A detailed systematic flowchart is shown in Fig. 1.

3.2. General description of data

Regarding the study overview, all relevant characteristics of the subjects, bioactive compounds and procedure are summarized in Table 1. The most common plant and bioactive compounds found were active principles derived from *Gymnema sylvestre*, used in 27 studies: gurmarin and gurmarin-related products, gymnemic acids and deacyl gymnemic acid. Other phytochemicals were extracted from *Hovenia dulcis* and *Ziziphus jujuba*. The method of administration included lingual and buccal application. Furthermore, application through both liquid and solid substances was utilized.

3.3. Study outcomes

Regarding the primary outcomes, 26 of the 28 studies reported only one of the outcomes (see Table 2). The remaining articles reported two outcomes, including eating behaviour and nutritional physiological status. Concerning the secondary outcome, only one

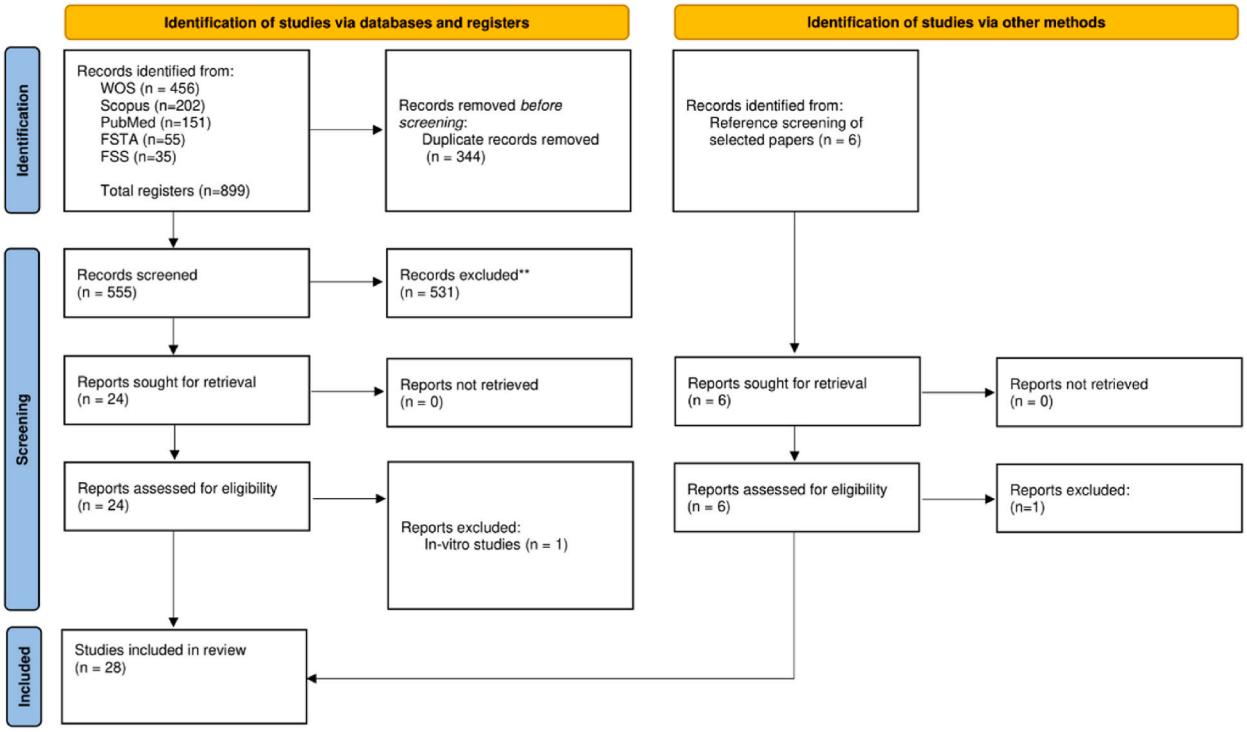

Fig. 1. PRISMA 2021 flow diagram and selection of original articles. Source: Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., … & Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. International Journal of Surgery, 88, 105906.

Table 1
Summary of biocompounds, animal's characteristics and procedure employed. Note: n.a.: not applicable; n.r.: not reported; w/v: weight/volume.

Biocompound/ plant (Supplier)	Specie (sample size)	Female percentage (%)	Age	Concentration	Oral administration (form)	Time administration	Days of treatment	Author (year)
Gurmarin (n.r.)	Rat (n.r.)	n.r.	n.r	10 μg/ml	Buccal (infusion)	10 min	n.a	Harada & Kasahara (2000)
Gurmarin (n.r.)	Rats/Sprague Dawley (n.r.)	0.0	n.r	10 μg/ml	Buccal (infusion)	n.r.	1	Lemon et al. (2003)
Gurmarin (n.r.)	Rats/Wistar (n.r.)	100.0	n.r	20 μg/ml and 5 mg/ml	Lingual (solution) and intravenous injection	10 min	n.a	Miyasaka & Imoto (1995)
Gurmarin (n.r.)	Mice/C57BL/KsJ (n.r.)	n.r.	8–25 weeks	30 μg/ml	Buccal (infusion)	1 min	n.a	Murata et al. (2003)
Gurmarin (n.r)	Mice/C57BL/6 (n.r)	n.r	n.r	30 μg/ml	Lingual (solution)	10 min	10	Nakashima et al. (2001)
Gurmarin (n.r.)	Mice/C57BL/ 6CrSlc (12) and BALB/cCrSlc (10)	45.4	n.r	1, 3, 10, 20, 50, 100 μg/ml	Lingual (solution)	10min	n.a	Ninomiya & Imoto (1995)
Gurmarin (n.r.)	Mice/C57BL/KsJ (28)	53.6	8–25 weeks	1, 3, 10, 20, 50, 100 μg/ml	Buccal (solution)	10min	n.a	Ninomiya et al. (1997)
Gurmarin (n.r.)	Mice/C57BL/KsJ diabetic (15) and non-diabetic (12)	0.0	8–20 weeks	1, 3, 10, 30, 100 μg/ml	Buccal (infusion)	10min	n.a	Ninomiya et al. (1998)
Gurmarin (n.r.)	Mice/C57BL/ 6CrSlc (36)	33.3	8–25 weeks	20 μg/ml	Oral administration (flow chamber)	5min	1	Ninomiya et al. (1999)
Gurmarin (n.r.)	Mice/C57BL/KsJ (18)	27.8	8–25 weeks	1, 3, 10, 30, 100 μg/ml	Buccal (infusion)	10min	n.a	Ninomiya, Inoue et al. (1998)
Gurmarin (n.r.)	Mice/C57BL/6 wildtype and T1R3-/G gust-/ TRPM5-KnockOut (n.r.)	n.r.	8–32 weeks	30 μg/ml	Lingual (solution)	10min	n.a	Ohkuri et al. (2009)
Gurmarin and derivatives (n. r.)	Rats/Wistar (n.r.)	0.0	n.r	10 μg/ml	Lingual (solution)	10min	n.a	Ota et al. (1998a)
Gurmarin and derivatives (n. r.)	Rats/Wistar (n.r.)	0.0	n.r	10 μg/ml	Lingual (solution)	10min	n.a	Ota et al. (1998b)
Gurmarin and pronase E (n. r.)	Rats/Wistar (35)	0.0	n.r	2%	Lingual (solution)	10min	n.a	Sako et al. (1994)
Gurmarin (n.r.)	Mice/129 × 1/ SvJ (12)	n.r.	8–25 weeks	30 μg/ml	Lingual (solution)	10min	n.a	Sanematsu et al. (2005)
Gurmarin (n.r.)	Rats/Wistar (10)	0.0	n.r	1, 3 and 10%	Buccal (solid food)	Unlimited	n.a	Yamada et al. (2006)
Gurmarin (n.r)	Mice/C57BL/ 6NCrj (n.r)	n.r	8–20 weeks	30 μg/ml	Lingual (infusion)	5 min	n.a	Yasumatsu et al. (2007)
Gurmarin (n.r)	Mice/C57BL/ 6NCrj (n.r)		8–20 weeks	30 μg/ml	Lingual (infusion)	5 min	n.a	Yasumatsu et al. (2009)
Gymnema sylvestre (Okinawa prefecture, Japan)	Rats/Wistar (n.r.)	100.0	n.r	10 μg/ml	Lingual (solution)	n.r.	n.a	Imoto et al. (1991)
Gymnema sylvestre (n.r.)	Rats/Wistar (n.r.)	0.0	n.r	1, 3 and 10%	Buccal (solid food)	Ad libitum	14	Katsukawa et al. (1999)
Gymnemic acids (Laboratories and Himalaya Drug Co., Bombay, Indie)	Hamsters (11)	0.0	n.r	0.025, 0.05, 0.1, 5 and 10%	Buccal and lingual (solution)	Unlimited	1	Faull & Halpern (1971)
Gymnemic acids (n.r.)	Hamsters (11) and rats (16)	n.r.	n.r	1, 2, 3, 5, 7.5 and 10 mg/ml	Lingual (solution)	3 min	n.a	Hellekant & Gopal (1976)
Gymnemic acid (n. r.)	Hamster (25)	0.0	1–6 months	5 mg/ml	Lingual (solution)	3 min	n.a	Hellekant & Roberts (1983)

(*continued on next page*)

Table 1 (*continued*)

Biocompound/ plant (Supplier)	Specie (sample size)	Female percentage (%)	Age	Concentration	Oral administration (form)	Time administration	Days of treatment	Author (year)
Gymnemic acids	Dogs (3), pigs (3) and rabbits (3)	n.r	n.r	1 mg/ml	Lingual (cotton soaked)	3 min	1	Hellekant (1976)
Gymnemic acids (n.r.)	Chimpanzees (9)	77.8	n.r	0.3, 1 and 3 mg/ml	Lingual (cotton soaked)	n.r.	2	Hellekant, Ninomiya et al. (1996)
Hodulcin (Arnold Arboretum of Harvard University)	Flies (28)	n.r	n.r	1% w/v	Labellar taste hair (solution)	2 min	1	Kolodny & Kennedy (1988)
Ziziphus jujuba (n. r)	Flies/*Phormia regina* (23)	n.r.	n.r	3.5% w/v	Labellar taste hair (solution)	3 min	1	Kennedy & Halper (1980b)
Ziziphus jujuba (n. r.)	Rats/Wistar, hamsters and human (n.r.)	100 (animals) and 0 (human)	n.r	n.r/0.2%	Lingual (solution)	n.r/3min	n.a	Yamada & Imoto (1987)

study examined the impact of sweetness inhibitors in specific populations, including genetically diabetic animal models and fructose-induced metabolic syndrome.

3.4. Taste perception in healthy animal subjects

3.4.1. Behavioural studies

Methods. 5 studies highlighted behavioural strategies to assess taste perception. For instance, in flies treated with *Ziziphus jujuba* and *Hovenia dulcis*, the suprathreshold stimulus was determined individually by stimulation of taste hairs with increasing sucrose concentrations at 15-min intervals until 3 consecutive positive (full proboscis extension) responses to particular concentrations were obtained [11,12].

Regarding hedonic methods for sweet tastes, two types of methodologies have been identified. On the one hand, the chimpanzees' liking after tasting the sweeteners was measured using observational coding by technicians on a six-point hedonic scale [13]. On the other hand, we found that other authors designed tests to minimize possible side effects of post-ingestive feedback on taste perception in C57BL mice. In particular, 2 rodent studies presented short-term sweet taste exposure after the application of a sweet-taste suppressor with licking microstructure analyses (numbers of licks per 10–30 s). With such pre-absorptive experimental designs, only taste but not visceral inputs were supposed to determine licking responses to sweeteners under a conditioned taste aversion paradigm [14] or with aversive bitter compounds dissolved in a strong sucrose solution [15].

Sweet stimuli. The inhibitory effects have been behaviourally confirmed on a wide range of sweeteners. These included saccharin (1.7 mM), sucrose (0.3 M), fructose (0.3 M), alitame (0.3 mM), cyclamate (10 mM), galactose (0.5 M) and glucose (0.5 M) in chimpanzees [13]; sucrose (5–250 mM in 50 mM NaCl) in flies [11,12]; and sucrose concentration (from 0.01 to 1.0 M in mixture with 1–3 mM quinine) in C57BL mice [14,15]. Nevertheless, the suppression of gymnemic acids varied across sweet items. For instance, Hellekant et al. [13] observed from complete abolishment (aspartame, saccharin) to about 50% reduction (xylitol) in chimpanzees.

Effectiveness across species. Experimental evidence revealed interspecies differences in the sweetness suppression effectiveness of different components extracted from *Gymnema sylvestre*. Thus, we identified effective treatments of gurmarin specifically in C57BL mice and Wistar rats [14,15], in opposition to gymnemic acids in chimpanzees [16]. Looking at the selective inhibition of gurmarin, Murata et al. [15] showed that this inhibitor selectively suppressed lick responses to sweeteners in C57BL mice, but not to quinine, NaCl or monosodium L-glutamate. In terms of concentration-response function for sucrose, the same study showed that treatment with 20 μl of gurmarin at a concentration of 10 μg/ml was ineffective for mixtures of lower (<0.03 M) or higher concentration (>1.0 M) of sucrose with 3 mM quinine. Likewise, gurmarin was found to suppress the avoidance of sucrose in C57BL mice [14]. In chimpanzees, higher concentrations of gymnemic acids (from 0.3 to 3 mg/ml) resulted in increased suppression, although 20% remained of the response to sucrose [13]. In the 2 studies examining other blockers, Kennedy and Halpern [11] and Kolodny and Kennedy [12] found that a 3.5% solution of extract of *Ziziphus jujuba* leaves and 1% w/v solution of an aqueous extract from *Hovenia dulcis* leaves suppressed sweetness perception in flies.

Temporal parameters. Effects of gurmarin have been observed to last 2–3 h in animal models [15]. In the case of ziziphin, the initial behavioural recovery times of sweetness suppression were reported to be within 2–6 min in the fly study [11,12]. Only one study assessed temporal parameters of hodulcine in fly behavioural tests, finding a median recovery time of 2 min [12].

3.4.2. Neural studies

Methods. Different nerves and neural structures have been assessed using electrophysiological procedures from single-fibre to whole-nerve recordings of action potentials evoked by taste stimuli after sweet-tasting suppressor treatment. Most of the 20 studies reviewed assessed the chorda tympani nerve in different species such as hamsters, rats, chimpanzees and mice [13,16–29]. Other

Table 2
Summary of outcomes, measures and significant results. Note: T: taste perception. E: eating behaviour. P: physiological status. "0" if the study failed to show a statistically significant difference ($p > .05$) and "+" if the biocompound tested achieved a significant difference ($p \leq .05$).

Outcome	Biocompound/ plant	Measure (unit)	Statistically significant differences	Author (year)
E	Gymnemic acids	Preference (% total intake)	+	Faull & Halpern (1971)
E/P	Gurmarin	Food intakes (g)/saliva (wet weights of submandibular glands [mg/100 g of body weight], protein concentration [mg/ml], toluenesulfonyl-L-arginine methyl ester activities of submandibular saliva [μmol substrate hydrolysed/ min/mg salivary protein])	+/+	Yamada et al. (2006)
E/P	*Gymnema sylvestre*	Food intake (g) and preference for sucrose mixtures (%)/saliva composition (relative weight of gland [%] and peroxidase activity [unit/mg protein])	+/+	Katsukawa et al. (1999)
T	Gurmarin	Electrophysiological response of superficial petrosal nerve (response magnitude, integrated response, percent response)	+	Harada & Kasahara (2000)
T	Gymnemic acids	Electrophysiological response of chorda tympani nerve (integrated response, percent response)	+	Hellekant & Gopal (1976)
T	Gymnemic acids	Electrophysiological of chorda tympani nerve (integrated response)	+/+	Hellekant & Roberts (1983)
T	Gymnemic acids	Electrophysiological response of chorda tympani nerve (peak response)	+ and 0	Hellekant (1976)
T	Gymnemic acids	Liking rating (6-point scale)/electrophysiological response of chorda tympani nerve (response magnitude and integrated response [impulses/sec], integrated response area, delay time [msec], steepness of the phasic response, time to reach the maximum response [msec], time needed for the activity to reach pre-stimulation level [msec])	+/+	Hellekant, Ninomiya et al. (1996)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (response magnitude and integrated response [impulses/sec]	+	Imoto et al. (1991)
T	*Ziziphus jujuba*	Proboscis extension response with suprathreshold stimulus/ electrophysiological response of labellar taste hair (integrated response [impulses/sec])	+/+	Kennedy & Halpern (1980b)
T	Hodulcin	Proboscis extension response/electrophysiological response of labellar taste hair (integrated response [impulses/sec])	+/+	Kolodny & Kennedy (1988)
T	Gurmarin	Electrophysiological response of nucleus tractus solitarii nerve (net spike/ sec)	+	Lemon et al. (2003)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec]	+	Miyasaka & Imoto (1995)
T	Gurmarin	Lick response (number of licks/sec)	+	Murata et al. (2003)
T	Gurmarin	Lick response (percent suppression score [number of licks/sec])	+	Nakashima et al. (2001)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec], percent response, relative response)	+	Ninomiya & Imoto (1995)
T	Gurmarin	Electrophysiological response of chorda tympani and glossopharyngeal nerves (response magnitude, integrated response [impulses/sec], percent response, relative response)	+ and 0	Ninomiya et al. (1997)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec], percent response, relative response)	+	Ninomiya et al. (1998)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (impulses/sec)	+	Ninomiya et al. (1999)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec], percent response, relative response)	+	Ninomiya, Inoue et al. (1998)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec], percent response, relative response)	+	Ohkuri et al. (2009)
T	Gurmarin and derivatives	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec])	+	Ota et al. (1998a)
T	Gurmarin and derivatives	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec])	+	Ota et al. (1998b)
T	Gurmarin and pronase E	Electrophysiological response of chorda tympani nerve (response magnitude, integrated response [impulses/sec])	+/+	Sako et al. (1994)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (integrated response [impulses/sec], relative response)	+	Sanematsu et al. (2005)
T	*Ziziphus jujuba*	Recognition threshold estimate (units of threshold change)/ electrophysiological response of chorda tympani nerve (integrated response [impulses/sec], relative response)	+/+	Yamada & Imoto (1987)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (integrated response [impulse/sec])	+	Yasumatsu et al. (2007)
T	Gurmarin	Electrophysiological response of chorda tympani nerve (integrated response [impulses/sec])	+	Yasumatsu et al. (2009)

studies examined the greater superficial petrosal nerve innervating palatal taste buds in rats [30], glossopharyngeal nerve in mice [31] or chemoreceptor neural responses in flies [11,12]. Lastly, one study focused on the central nervous system, specifically on the gustatory-responsive portion of the nucleus of the solitary tract in rats [32]. Quantitative parameters of neural responses to sweetener included the number of recordings, maximum amplitude of response, integrated response, delay time in milliseconds between the onset of stimulation and beginning of response, steepness of the phasic response, time in milliseconds to reach the maximum response, magnitude of the activity after stimulation, and time in milliseconds needed for the activity to reach the pre-stimulation level during rinsing after stimulation [13]. Response magnitude and integrated response were among the most used techniques [11,12,14,16–20,22–31,33,34].

Sweet stimuli. In animals, mostly the neural response to basic sweet taste was obtained from sucrose, fructose, glucose, maltose, saccharine and amino acids. In the case of gymnemic acids, 4 studies assessed substances with sweet potencies about 0.3–0.5 M sucrose, 0.5 M fructose and glucose in pigs, dogs and rabbits; 0.01 M acesulfame, 0.6 M fructose, 0.8 M xylitol, 1.0 M glucose, 0.03–0.3 M sucrose and 0.03–0.15 M saccharin in hamsters; and 4.3 mM acesulfame, 0.3 mM alitame, 5.1 mM aspartame, 10 mM cyclamate, 0.5 M galactose, D-glucose, mannose, 1.7 mM saccharin, 0.3 M sucrose, fructose, 0.9 stevioside and 0.8 M xylitol in chimpanzees [13,16,18,35]. Gurmarin had essentially no effects on responses to polycose in rats [34].

Effectiveness across species. Regarding interspecies differences, the neural responses to sucrose, aspartame and/or saccharin were suppressed by 1–3 mg/ml gymnemic acids in chimpanzees [13], dogs [35] and rats [35]. However, similar or higher concentrations caused no consistent decrease in responses to these sweeteners in other primates, pigs, rabbits and hamsters [18,35]. In addition, 2 studies assessed a concentration-response relationship, one in chimpanzees and another one in hamsters. In the first one, Hellekant et al. [13] found that the strongest suppression was exerted by the highest assayed concentration (from 0.3 to 3 mg/ml). In the second one, depression of the hamster response to sucrose was directly related to the strength of the gymnemic acids: 1–2 mg/ml caused a slight general suppression of the response in 2 out of 7 hamsters tested; 3–5 mg/ml depressed the response to all stimuli in 3 out of 5 animals and 7.5–10 mg/ml in 5 out of 5 hamsters [16].

In rats, 8 studies showed that gurmarin suppressed neural responses not only to sugars (sucrose, glucose, fructose and maltose) but also to sweet amino acids and saccharin, without affecting responses to salty, sour and bitter substances and non-sweet amino acids in rat and mouse taste nerves [19,20,24,25,29–31,33]. Gurmarin concentration equal to or higher than 10 μg/ml markedly suppressed neural responses to sugars by 40–50% from greater superficial petrosal nerve [30], 57-33% from the solitary nucleus [32] and from the chorda tympani nerve [24,34]. Focused on the chorda tympani, Miyasaka and Imoto's [20] study showed that even 20 μg/ml gurmarin was sufficient to show its maximal effect on sugars, sweet amino acids and saccharin in rats.

In mice, 3 electrophysiological studies [23,29,33] demonstrated that sucrose responses of the chorda tympani innervating taste buds located in the anterior two-thirds of the tongue were suppressed to ~50% of control by gurmarin in C57BL mice. Moreover, the effect of ≥3 μg/ml gurmarin on the tongue for 10 min selectively suppressed neural chorda tympani responses to sweeteners by about 45–75%, without affecting responses to NaCl and quinine [30,33]. By contrast, only a slight tendency for concentration-dependent reduction of sucrose responses was observed in BALB mice. In particular, the BALB strain neural responses were hardly affected, even with gurmarin at 100 μg/ml, showing a difference in the threshold of gurmarin concentration for inhibition of sucrose response >34 times higher than in C57BL mice [30,31]. Unlike chorda tympani, responses of the glossopharyngeal nerve innervating taste buds located in the posterior one-third of the tongue were not affected by gurmarin in C57BL mice [33].

Finally, one study showed a suppressive chorda tympani effect of sucrose responses by 0.5% solutions of different fractions obtained from *Ziziphus jujuba* and applied to the tongue for 5 min in rats and hamsters [17]. Two other studies with flies reported that labellar chemoreceptor firing rates to sucrose decreased after a 3-min treatment with 3.5% extract of *Ziziphus jujuba* [11] or after a 2-min treatment with 1% extract from *Hovenia dulcis* [12].

Temporal parameters. Out of the 23 electrophysiological studies, only in 7 did researchers examine the specific time course of sweet inhibition and recovery. In 2 of these studies, complete recovery of the suppressed rat neural responses required at least 3 h after gurmarin treatment, with no differences among sweeteners like sucrose, glucose, fructose or saccharin [19,20]. However, not all studies have found similar recovery patterns among sweeteners. In mice, the recovery time course to fructose and saccharin started within 30 min after gurmarin treatment, and over 1 h to sucrose and glucose, while full recovery ranged from 1 h (fructose) to 2 h (glucose), in the following order: fructose > saccharine > sucrose > glucose [29]. It should be noted that the effect of gurmarin was not instantaneous after application, requiring around 5 min to produce maximal suppression of sweet responses recorded from single mouse fibres [21,32].

In the case of ziziphin, the mean recovery times were reported to be within 30 min in rats and hamsters [17] and 10 min for hodulcin in flies [12]. In flies, researchers have found that ziziphin treatment decreased flies' labellar chemoreceptor firing rates to sucrose, with a median reduction of 60% at 1 min posttreatment and a gradual increase in response magnitude to 100% of preziziphin values at 7 min posttreatment [11].

3.5. Eating behaviour in healthy animal subjects: consumption and preference

Methods. Animal studies approached eating behaviour by measuring changes in acceptance tests with one-bottle or in flavour preference with two-bottle (sweetener versus water) choice tests. For instance, the one-bottle technique before and after gymnemic acids was used in chimpanzees [13]. In the case of preference, this was calculated as a percentage of sweet intake relative to the total volume consumed. A key strength of the preference studies is that they allow determining whether a taste stimulus is preferred over water and to discriminate the impact of body fluid homeostasis and taste and/or oropharyngeal factors in sweet consumption since the need for water may be accomplished by drinking water. Preference was obtained for hamsters [36] and rats [37]. Additionally, total

food intake was measured during a week by Yamada et al. [38].

Sweet stimuli. Only sucrose was included in studies with hamsters (from 0.02 M to 0.25 M) [36]; or sucrose (0.01–0.03 M) and mixtures of sucrose and quinine in rats [38].

Effectiveness and temporal parameters. No differences in daily intake were found in rats repeatedly fed a diet containing *Gymnema sylvestre* (3% gurmarin-containing diet at a concentration of 0.1 μg/ml). Notwithstanding, they exhibited a transient reduction in preference for 0.01 M sucrose and a mixture of 0.03 M sucrose and 0.03 M quinine (13.2% and 23.3%, respectively, compared to the control levels) at 1–2 days after the start of the diet, subsequently returning to control levels within a week. This change in preference was not observed for higher concentrations of sucrose (0.03 M) or other no-sweet taste solutions [38].

3.6. Physiological status in healthy animal subjects

Methods. In 2 studies researchers investigated saliva-related changes that may modify the sweet-suppressing effect of gurmarin in rats with diets containing 1% [38] or 3% *Gymnema sylvestre* [37]. To do so, collection of submandibular saliva was conducted.

Effectiveness and temporal parameters. Regarding the temporal induction of salivary proteins, a *Gymnema sylvestre* diet for 4 or 14 days increased the inhibitory gurmarin effect of saliva of rats compared with the control-diet group [38].

In the 2 studies examining the involvement of oral sensory system for salivary protein induction in healthy animals, no differences in the relative weight of the salivary glands were found by either Katsukawa et al. [37] or Yamada et al. [38] in animals fed either the control or the *Gymnema sylvestre* diet. No differences were found either in the salivary flow rate. Notwithstanding, Katsukawa et al. [37] observed a rise in gurmarin-binding proteins in saliva of rats fed a 3% *Gymnema sylvestre* diet. In a subsequent study, Yamada et al. [38] reported that these salivary proteins blocking gurmarin were rat kallikreins 2 and 9, which are suggested to be a line of defence against unfavourable reduction in sensitivities to sweet substances.

3.7. Results in specific populations

Only one study examined the impact of sweetness inhibitors in specific populations. In the context of genetically diabetic animal models, leptin receptor-deficient db/db mice were used [22]. Although chorda tympani responses to all sweeteners tested (sucrose, saccharin, glycine, L -alanine, and D -tryptophan, but not to D-phenylalanine) were suppressed by 100 μg/ml gurmarin, the degree of suppression was smaller and the threshold of gurmarin inhibition was higher in the diabetic than in the control mice.

4. Discussion

This systematic review aims to investigate the preclinical effects of sweet taste blockers on taste perception, eating behaviour and physiological status from a preclinical perspective. As widely recognized in the literature, our analysis revealed empirical evidence of sweet taste modification in three plants (*Hovenia dulcis*, *Ziziphus jujuba*, and *Gymnema sylvestre*). The studies focused primarily on taste perception and utilized neurophysiological techniques. We found that extracts of *Gymnema sylvestre* and its derivative gurmarin consistently suppressed both electrophysiological response and sweet food consumption and preference. Similarly, *Ziziphus jujuba* and *Hovenia dulcis* produced comparable results, albeit less frequently represented in the studies reviewed. Given that most of these studies were primarily aimed at unravelling the physiological mechanisms of sweet taste receptors, there was a lack of prior research that comprehensively examined the impact of diminished sweet taste sensations on feeding behaviour and, consequently, the nutritional status of animals. As a result, the downstream effects of sweet taste perception on short-term energy intake and long-term weight status remain to be investigated.

4.1. Mechanisms of sweetness inhibition of bioactive compounds: from molecules to eating behaviour

According to the preclinical studies, anti-sweet compounds may interact with different binding sites on sweet receptors. However, little is known about the mechanisms underlying such differential sweet taste inhibition. In taste tests, extracts and principles from *Gymnema sylvestre*, *Hovenia dulcis* and *Ziziphus jujuba* exhibited a certain level of selectivity. However, it is uncertain how different classes of sweeteners may potentially compete with these bioactive compounds at the receptor level. As to *Gymnema sylvestre*, some authors have suggested the competitive account to explain why the taste of sucrose is not immediately affected by the pharmacological action of *Gymnema sylvestre*. In particular, such a delayed effect could represent a gradual action on the receptor membrane by either displacing the sucrose stimulus from its sites or by occupying sites when they are vacated [39]. However, the most supported hypothesis was the non-competitive inhibition of the transduction process [40,41]. In opposition to the diminished inhibition with increasing sweetener concentration of the competitive binding account, Frank et al. [44] and Riskey et al. [43] found a constant percentage reduction of the subjects' perceived sweetness intensity produced by gymnemic acids regardless of sweetener concentration. In a similar non-competitive way, Smith and Halpern's study [40] showed selective taste modifications of sweetness, even when the inhibitor was presented simultaneously with the mixture of sugars. Nevertheless, the lack of further evidence to rule out competitive and non-competitive mechanisms [39,44–46] has also led to posit presence of a concentration-dependent mechanism [42]: a high-concentration extract produced non-competitive inhibition, while a low-concentration extract produced competitive inhibition.

Regarding the biological processes behind sweet-taste-suppressing substances, sweet-taste blockers such as gymnemic acids and ziziphin appear to act on transduction processes in taste receptor cell membranes. Specifically, with regard to the sweet taste receptor

T1R2–T1R3, multiple binding sites for anti-sweet agents have been proposed. One line of evidence came from differential inhibition effects: unlike *Ziziphus jujuba*, *Gymnema sylvestre* eliminated not only the sweet sensation elicited by natural and artificial sweeteners, but also the sweet amino acids in humans [47]. For lactisol, *in vitro* studies at the receptor level showed that it may act as a competitive inhibitor of the sweet taste. The binding site seems to be located on the T1R3 subunit since lactisol also interacts with the umami taste, but as a non-competitive inhibitor [48].

The fact that multiple receptor sites are involved is also supported by interspecies differences, even in mammalian species. Ninomiya and Imoto [29] reported that the sweet neural responses observed in BALB strain mice were hardly affected by the application of gurmarin, while they were suppressed in the case of the C57 BL strain. Subsequent studies have suggested the existence of both types of gurmarin-sensitive and gurmarin-insensitive receptors for sweeteners in C57BL mice, which are differentially distributed in the mouth [21]. The fact that sweet taste perception varies across species also suggests the existence of phylogenetic differences in the mechanism of sweet taste recognition [19], with multiple ways of sweet taste transduction developed through evolution. The most striking example is the case of gymnemic acids that did not affect the taste response to sucrose in rabbits or rats, but largely suppressed the sweet taste sensation in hamsters, chimpanzees and humans. Moreover, gurmarin is a protein that also comes from *Gymnema sylvestre* and suppresses sweet taste perception in rodents, but not in humans or primates [13,16,18,35]. Nevertheless, differential mechanisms of inhibition remain undetermined, perhaps due to the lack of knowledge of the structure of the T1R2/T1R3 receptor. To date, much remains to be determined regarding its molecular structure, the mechanisms of sweetener binding and the mechanisms of receptor activation [49,50].

Interestingly, mouse strain differences in sensitivity to the sweet-suppressing effect of gurmarin may not be associated with polymorphisms in *TAS1R3*. Using 129 mice with non-taster *TAS1R3* allele, Sanematsu et al. [26] assessed the effect of gurmarin on chorda tympani responses to sweet compounds. Their results indicated that this non-taster strain clearly showed chorda tympani inhibition to various sweet compounds similar to that observed in C57BL mice having the *TAS1R3* taster genotype. Moreover, Ohkuri et al. [23] demonstrated that responses to sucrose and glucose, but not to saccharin, were gurmarin-sensitive in T1R3-knockout mice.

Focused on the gurmarin-sensitive pathway, animal research has shown that the receptor-interacting site of gurmarin is not the basolateral part, but the apical side of the taste cell. Indeed, 4 studies demonstrated that the anti-sweet effects of gurmarin rapidly disappeared after rinsing with either *anti*-gurmarin serum in rats [19,20] or β-cyclodextrin in C57BL mice [29,33]. In another study, researchers found strong efficacy of lingual application of gurmarin on the response to sucrose vis-à-vis the lack of detectable change by intravenous injection [20].

Selective synaptic coupling between taste receptor cells that express gurmarin-sensitive or insensitive receptors and particular subsets of sucrose-best chorda tympani fibres have been proposed [32] and supported by a series of studies led mainly by Ninomiya et al. As an example, Ninomiya et al. [21] highlighted that gurmarin inhibited some but not all mouse neural responses to sweet compounds, in particular, only a subset of sucrose-best chorda tympani fibres in C57BL mice. Thus, input arising from gurmarin-sensitive and gurmarin-insensitive sweet receptors and transduction mechanisms seem to be also segregated into particular classes of sweet-responsive chord tympani fibres in C57BL mice. In particular, the lingual application of gurmarin in this mouse strain attenuated some responses of the chorda tympani nerve and the greater superficial petrosal nerve responses to sweet-tasting substances [22,29,30], but without effect on the glossopharyngeal nerve responses [31].

The neural structure of the central nervous system that receives gustatory information is the nucleus of the solitary tract. Information from gurmarin-sensitive and insensitive receptors [32] converge onto this nucleus, where inputs from other taste nerves also are integrated. The inhibition of the chorda tympany does not produce the corresponding behavioural response due to the neural integration of different sources [31].

Finally, the temporary reduced sensory neural input treatment with *Gymnema sylvestre* has been associated with a decrease in pleasantness, desire and consumption of sweeteners in humans [51]. In this sense, Stice and Yokum [52] used fMRI and showed that blocking human sweet taste receptors with gymnemic acids not only reduced the reward region response to the intake of high-sugar foods, but also reduced anticipated reward from high-sugar foods, potentially via a feedback loop regarding the availability of sweet taste receptors to convey perceptual input concerning sweet tastes.

4.2. Limitations

We have observed that the *Gymnema sylvestre* compounds produce species-specific results. This could also be the case with other sweetness inhibitors already tested in humans but not in animals such as *Stephanotis lutchuensis, Gymnema alternifolium* or *Styrax japonica* [53–58]. This may also include *Aesculus Hippocastanum* which has been mentioned as a sweetness suppressor, but actually their effects are already unknown in animals and humans [59]. In this sense, several challenges exist in respect of the use of preclinical animal models such as limited translatability and generalizability when taking into account the species-specific results. It becomes difficult to accurately predict the effects in humans based on data from a single animal model. This can hinder applicability and effectiveness of preclinical research in guiding clinical trials and developing treatments for human use. Moreover, the safety profiles of compounds or interventions may differ between species, which can complicate the process of evaluating potential risks and benefits. Thus, it is recommended to conduct preclinical studies with species-specific results and additional resources, including a broader range of animal models, in order to validate and verify findings, as well as other complementary research methods, such as *in vitro* studies or computational modelling.

Methodological limitations arise from sample size, which varies from 3 to 36 subjects and is not reported in 13 of the studies (43.3%). On the other hand, even though the aim of this review was to explore the effects of sweet taste inhibitor compounds on three different levels (perception, behaviour and physiology), we found a large bias because 25 of the studies (83.3%) only reported results

about taste perception. Additional issues relating to the methodological design of the behavioural animal experiments should be noted: 1) disparate animal species and strains (mice, chimpanzees, flies); 2) variations in concentration and/or doses; 3) lack of control groups; 4) no methods for blinding investigators, either acknowledged or reported; and 5) small sample size with inadequate statistical power when reported. For instance, 20% of studies had a total sample size of $\leq$11 subjects [13] while 40% did not report the sample size [14,15].

In particular, the studies included in this review record all the main plants studied in the field of sweetness inhibition. However, two of the outcomes, perception and physiological status, have only been studied with *Gymnema sylvestre* and derivatives [36–38]. The limit already mentioned between the neural response of tasting and behaviour correlations remains unclear. Chorda tympani nerve inhibition appears clearly throughout the studies while the intake or preference reduction has not been sufficiently studied. Then, only three different papers have studied the electrophysiological response of a nerve other than chorda tympani, but not at the same time [30–32]. Integration among the nerves implicated in sweet perception continues to be unclear.

The temporal profiles (specifically treatment and stimulus durations and recovery time) varied across the stimuli and the blockers, with shorter recovery times in the sweetness effects of ziziphin and hodulcine compared to gymnemic acids. Nevertheless, in order to further understand such differences, further research is still required on phenomena occurring with the sweet blockers during their transit through the mouth (such as diffusion rates and chemical reactions), as well as those directly involving the receptor (such as sites for ligand recognition, taste receptor molecular behaviour, affinities and structural rearrangements). Furthermore, the relationship between inhibition of the sweet-taste receptor and the behavioural changes in sugar selection and consumption is not simple or direct. For example, it may be mediated by the integration and processing of orosensory inputs in central gustatory circuits such as the nucleus of the solitary tract [32] or by the existence of an alternative pathway to sense sugars independent of the canonical T1R2-T1R3-mediated pathway [60]. Consequently, more studies are necessary.

None of the reviewed studies included the synergic/additive blocking effects of two inhibitors administered simultaneously on sweetness perception. In this sense, if two blockers act via the same mechanism and compete for the same binding site, it is possible for them to behave in an additive way. Conversely, if two blockers have separate orthostatic or allosteric binding sites, they may have a synergic/cooperative binding effect. Therefore, more research using sensory experiments is required to further explore this effect.

4.3. Conclusions

Our review examined the current literature on the effects of natural sweetness inhibitors on behavioural, neural and physiological outcomes, with a focus on the impact of attenuating oral sweet sensations. We identified three plant species, *Gymnema sylvestre*, *Hovenia dulcis* and *Ziziphus jujuba*, which possess acute sweetness-inhibitory properties. When administered orally, these plants acutely reduced neural responses to sweet stimuli and decreased within-session consumption. Unfortunately, no studies were found that concurrently examined the effects of reduced sweet taste sensations on feeding behaviour and, ultimately, the nutritional status of animals. Furthermore, it will be crucial to make a clear distinction between the short-term within-session effects (i.e., refraining from consuming additional sugar now) and the longer-term effects (i.e., decreasing the overall consumption of sugar in the future). On the other hand, in preclinical animal models, different components extracted from *Gymnema sylvestre* have varying levels of sweetness suppression effectiveness and these levels may differ among different species. This variability must be taken into account when selecting an animal model to study sweetness inhibition in humans. Finally, further research using specific and highly controlled designs is needed to evaluate the therapeutic applications of these sweetness inhibitors. Such research would help determine the effectiveness of these inhibitors in fighting global added sugar consumption and associated health issues, as well as their potential use as a therapeutic agent in eating- and weight-related problems.

CRediT roles

All authors listed have significantly contributed to the development and the writing of this article. Raquel Rayo-Morales: analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper. Antonio Segura-Carretero: analyzed and interpreted the data. Maria Isabel Borras-Linares: analyzed and interpreted the data. David Garcia-Burgos: conceived and designed the review; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Raquel Rayo Morales reports financial support was provided by Government of Spain Ministry of Universities. David Garcia-Burgos reports financial support was provided by Spain Ministry of Science and Innovation.

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