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Modulation of sugar feeding behavior by *Gymnema sylvestre* in *Drosophila melanogaster*

Shivam Kaushik¹, Rahul Kumar², Sachin Kumar³, Srishti Sanghi⁴, Pinky Kain¹

¹Regional Centre for Biotechnology, Faridabad, Haryana, India

²University of New Mexico, USA

³Viresco technologies LLP, Roorkee, India

⁴Department of Bioscience and Bioengineering, Indian Institute of Technology Jodhpur, India

Abstract

Introduction—Sugar is the main source of energy for nearly all animals. However, consumption of a high amount of sugars can lead to many metabolic disorders hence, balancing calorie intake in the form of sugar is required. Various herbs are in use to control body weight, cure diabetes and control elevated blood sugar levels. One such herb is *Gymnema sylvestre* commonly called Gurmar (destroyer of sugar). Gurmar selectively inhibits sugar sensation by mechanisms that are still elusive.

Objectives—The primary objective of this study is to understand the effect of gurmar on sweet taste feeding behaviour in insects using the invertebrate model system *Drosophila melanogaster*.

Methods—For this study, we used feeding assays, spectrophotometry and Proboscis Extension Reflex (PER) assay to determine how flies detect gurmar. Additionally, life span analysis, egg-laying behaviour and developmental profiles were used to probe the role of gurmar on the overall health of the flies. During the whole study, we used only the raw powdered form of gurmar (dried leaves) to examine its effect on sweet taste feeding behaviour.

Results—Our data demonstrate that whole gurmar in a raw powdered form is aversive to flies and inhibits sugar evoked PER and feeding responses. Also, we observed it takes at least 24 h of starvation time to reduce the consumption of sugar in flies pre-fed on gurmar. Flies lay a fewer

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Correspondence to: Pinky Kain.

Corresponding author: Pinky Kain, Neurobiology and genetics, Regional Centre for Biotechnology, NCR Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad - 121 001, Haryana (NCR Delhi), India. pksharma@rcb.res.in.

Author contributions

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number of eggs on gurmar media and show developmental defects. Our data suggest that flies detect gurmar using both taste and olfactory cues.

Conclusion—Understanding how gurmar reshapes taste curves to promote reduced consumption of sugars in flies will open up avenues to help people with health issues related to high sugar consumption, but our data also highlights that its consumption should be carefully considered since gurmar is aversive to flies and has detrimental effects on development.

Keywords

taste; *Drosophila* ; sugar; modulation; starvation

Introduction

Humans have an innate liking for sweet taste. Sugars are the main source of energy for almost all living animals. Morbidities caused by the consumption of high amounts of sugar are increasing at an alarming rate, creating a huge economic burden on society and health care systems. In the current world, metabolic disorder- Diabetes is one of the major health threats that causes prolonged ill health, making it one of the leading causes of global mortality. In the absence of preventive measures, around 366 million people worldwide are expected to have diabetes by 2030.¹

Around 80% of the total world's population still use and rely on traditional medicine including phytochemicals from plants of medicinal importance for their primary health care and to treat various diseases.²⁻⁶ One such herb is *Gymnema sylvestre* commonly called as Gurmar (destroyer of sugar)⁷ to treat Diabetes mellitus which is caused by the presence of excessive sugar (glucose) in the blood. It has been shown that the chewing of gurmar leaves can cause a temporary loss of the sweet taste sense and curbs the cravings for sugar for a few hours.⁷⁻¹⁰ The anti-sweet principle in gurmar was found to be 'gymnemic acid'.¹⁰ The anti-sweetness effect of gymnemic acid varies from species to species, even in mammals.¹¹⁻¹³

In both animals and humans, gurmar increases urine output and reduces hyperglycemia.¹⁴ Hot water extract from the leaves of *G. sylvestre* causes suppression of sweet taste responses in the rat.¹⁵ The inhibitory effect of another chemical-gurmarin, was found to be highly specific to the sweet taste response to sucrose, glucose, glycine and saccharin with no effect on salt (NaCl), sour (HCl) and bitter taste (quinine).¹⁶ Gurmarin present in leaves of *G. sylvestre* is a polypeptide that consists of 35 amino acid residues including three intramolecular disulfide bonds.¹⁶ Although gurmarin suppresses the sweet taste response in rats, a weak or no effect was seen in human beings.¹⁵⁻¹⁷ The absence of available consolidated scientific data for the antidiabetic effect of *G. sylvestre* from clinical trials calls for the examination of its mechanism of action and the understanding of its effects on sugar taste sensation in animals.

Artificial sweeteners, sugar concentrations ranging from 100–500 mM and various sweet tasting proteins elicit sweet taste in mammals. The T1R2 and T1R3 heterodimer constitute the sweet taste receptor in mammals.¹⁸ Flies also get attracted to many of the sugars that

humans consume.^{19,20} *Drosophila melanogaster* is one of the ideal genetic systems to study feeding behaviour. Flies are able to detect and differentiate sugars, bitter substances and other gustatory cues through various taste sensors present on different taste organs. These compounds produce an attractive or repulsive response in behavioural tests that finally leads to acceptance or rejection of food.^{21,22} 60 genes in the gustatory receptor (GR) gene family encode 68 receptor proteins in *Drosophila*.^{23–25} The three key gustatory receptors required for sensing sugars (except fructose) in *Drosophila* are Gr5a, Gr64a and Gr64f.^{26–30}

To understand how gumar modulates feeding behaviour in insects, we used *Drosophila* as a model system to probe its effect on the sugar feeding behaviour in flies. Our feeding and PER data demonstrate that flies show partial (feeding assays) and almost complete repulsion (PER assays) to different concentrations of gumar. Flies lay a fewer number of eggs on gumar media plates. We also observed developmental defects and developmental delay in wildtype flies when grown on gumar media. We observed that a long starvation time is required after gumar consumption in flies to suppress sugar intake behaviour. Our data suggest that flies detect gumar using both taste and olfactory cues.

Results

Wildtype flies show aversive behaviour to gumar

To understand how flies respond to gumar, we first tested wildtype (*CsBz*) flies for their feeding preferences in a feeding assay plate (Figure 1A). For examining the feeding behaviour in feeding assay, batches of flies (20 flies each plate, 10 males + 10 females) were presented with a choice between water and varying concentrations of whole gumar (2.5%, 5% and 10% raw powdered form dissolved in water). We observed gumar dry leaves powder does not dissolve completely in water. The same concentrations of gumar were also tested after mixing it with sucrose (50 mM) and compared with 50 mM sucrose alone (control) (Figure 1B).

We found ~ 67% mean feeding responses for 50 mM sucrose. Wildtype flies showed feeding responses of ~ 45% for 2.5% gumar (with and without sucrose). We observed ~ 10–15% feeding responses for 5% and 10% gumar and between 30–40% for 5% and 10% gumar mixed with 50 mM sucrose (Figure 1B). Nonetheless, in all the tested concentrations of gumar with and without sucrose, we found significant differences when compared to sucrose alone (Figure 1B). We also found significant differences between gumar alone and gumar mixed with sucrose at 5% and 10% concentrations (Figure 1B, black and gray bars at 5% and 10% concentration). Since the feeding responses of 10% gumar and 5% gumar were not found to be very different from each other and showed a clear aversive behaviour (Figure 1B) later, we used both 5 and 10% gumar for the rest of the experiments. Our feeding results were confirmed again by measuring the food consumption via spectrophotometry (Figure 1C) separately. The absorbance values (Figure 1C) for 5 and 10% gumar were found to be reduced compared to 50 mM sucrose alone. We observed a significant difference between 5% gumar + sucrose and sucrose alone as well in spectrophotometry data (Figure 1C). Our data suggest that gumar is aversive to flies.

Gurmar is detected by both taste and olfactory cues

To investigate how flies detect gurmar, the main olfactory organ- antennae of the flies were removed. Antenna-less wildtype flies were tested for their feeding behaviour. In the absence of antennae, flies feeding on 5% whole gurmar showed improved and increased feeding responses after starvation in feeding assays when compared to wildtype control flies with intact antennae but not in the case of 10% gurmar (Figure 1D and E, red bars). The feeding responses for 10 and 5% gurmar + 50 mM sucrose were found enhanced as well in case of antennae less flies (Figure 1D and E, gray bars) in comparison to wildtype control flies. Our data suggest that the presence of the main olfactory organ in flies caused further repulsion as seen in control flies (Figure 1B and C). Overall, we found aversion to both 5 and 10% gurmar with and without 50 mM sucrose decreased and feeding responses improved after removing antennae. Our results support that feeding on gurmar with and without sucrose is dependent on both olfaction and taste (Figure 1D and E).

The feeding responses of 5 and 10% gurmar alone were less than 20%, we used these two concentrations of gurmar with the increasing concentration of sucrose to test the inhibitory effect of gurmar on sugar feeding behaviour. In our dose-response curves, we observed an increase in mean feeding responses with the increase in sucrose concentrations (Figure 1F). A similar pattern of feeding was observed when sucrose was mixed with 5 and 10% gurmar concentration. Highest responses were observed at 5 and 10% gurmar mixed with 100 mM sucrose (Figure 1F). We found significant feeding differences between sucrose and gurmar mixed with sucrose at all the tested concentrations (except 100 mM 10% gurmar). Our feeding assay results suggest inhibition of sugar feeding in the presence of gurmar (Figure 1F) and gurmar inhibits sucrose provoked feeding responses.

To confirm our feeding assay results, we also performed PER (Proboscis Extension Reflex) assay (Figure 2A). Extension of the proboscis is the first step shown by flies in an appetitive behaviour. Compared to 50 mM sucrose (~60% positive PER responses), wildtype flies showed less than 5% response when 2.5% and 5% gurmar were tested in our tarsal PER assay (Figure 2B, black bars). We didn't see any extension of proboscis at 10% gurmar. Significant differences in PER responses (34%, 26% and 21%) were observed when gurmar mixed 50 mM sucrose was compared with 50 mM sucrose alone (Figure 2B, blue bars). Our PER results suggest gurmar alone acts as an aversive cue to wildtype flies and inhibits sucrose evoked PER responses (Figure 2B).

Whole gurmar solution is more aversive to flies than just the extract present in the liquid fraction

In our feeding assays, a powdered form of dry gurmar leaves mixed with water and agar was presented to flies. We always observed settled solid particles at the bottom and green liquid solution at the top, suggesting gurmar doesn't completely dissolve in water. To determine which part of the gurmar solution is aversive to flies, we tested wildtype flies in feeding assays. Flies feeding on the liquid fraction of 10% gurmar + 50 mM sucrose and 5% gurmar + 50 mM sucrose showed improved feeding responses (65–75%) compared to whole gurmar at 10 and 5% concentration when mixed with 50 mM sucrose (40–45%, Figure 2C and D).

We also tested flies with 10 and 5% gurmar alone without adding any sucrose (Figure 2C and D) as a negative control. Just like in the case of Figures 1B and C, we again found similar feeding results (<20%) for 10 and 5% gurmar. In the absence of any sucrose, flies feeding on the liquid fraction of 10 and 5% gurmar alone showed ~45% feeding response as seen for 10 and 5% whole gurmar when mixed with 50 mM sucrose (Figure 2C and D). We found a few flies feed on whole gurmar without any added sucrose. Our results suggest that whole gurmar affects feeding behaviour more strongly and is highly aversive in nature, suggesting the presence of an aversive component in the solid particles rather than the liquid fraction.

Effect of gurmar on life span and egg-laying behaviour

Our results suggest gurmar is an aversive cue to flies, we further investigated the effect of gurmar on the development of flies. For probing the effect of gurmar on fly development, first, we tested the effect of gurmar on the egg laying behaviour of flies. Egg-laying was performed on the normal media and gurmar plates (gurmar mixed with normal media). Flies on the gurmar plates laid less number of eggs (on 5% - ~110–130 eggs and on 10% - ~ 80–100 eggs) compared to normal media flies (~150–170 eggs) (Figure 3A). We also observed that on 5 and 10% gurmar media, the mean number of flies eclosed per vial was less compared to normal media vials (Figure 3B). Our data suggest that gurmar feeding affects the egg laying behaviour in flies that resulted in reduced counts of eggs laid by flies. Altogether our data suggest that gurmar affects the egg-laying behaviour and hence, the total number of eggs laid.

We also determined if flies raised on gurmar throughout the development show any developmental defects. We raised the flies at 25°C in the incubator throughout the development. We noticed, the growth of the flies was slower and the flies eclosed late in the case of 5 and 10% gurmar (gurmar mixed with normal food) (Figure 3C) compared with normal media flies (NM vials). Flies growing on normal media eclosed on day 10–11 at 25°C (Figure 3C). On 5% gurmar, flies eclosed on 13–14th day and on 10% gurmar, flies eclosed as adults on day 17–18 (Figure 3C). We also observed that the size of the flies (both male and females) in the case of 10% gurmar was smaller compared to normal media raised (NM) flies (Figure 3D-D’). No differences in body size of flies were observed at 5% gurmar (Figure 3D’ and D’). Our data suggest that continuous consumption of gurmar causes deleterious effects on the development and overall health of the flies.

We further investigated the effect of gurmar feeding on the life span of flies. The longevity assay was used to follow the life spans of flies till death. Adult wildtype flies were transferred on the fresh gurmar media (gurmar mixed with normal fly media) and control media every alternative day to avoid any fly death due to soggy media conditions. The flies on 5 (green line) and 10% (red line) gurmar showed shorter life spans compared to normal media flies (65 days) (Figure 3E, blue line). Flies on 10% gurmar died at day 18 and on 5% at day 41 compared to normal media flies which died on day 65. Our data suggest continuous feeding on gurmar causes early lethality in wildtype flies compared to normal media flies (Figure 3E).

Effect of gurmar on sugar intake in flies

To test the effect of gurmar on sugar intake in flies, wildtype flies were fed for 2 days with 5 and 10% gurmar mixed with the normal media. Later flies were tested for their consumption of 50 mM sucrose in our 2 h feeding assay. Flies on normal media without any gurmar were used as a control. Two sets of flies were tested; one set was tested after 24hrs starvation and the other set was tested without any starvation (no starvation). Under no starvation condition, normal media wildtype flies consumed less sugar (~20–30% feeding response; Figure 4A, C and E) compared to 24 h starvation (~ 80–90% feeding response) flies on normal media (Figure 4B, D and F).

In case of both 10 and 5% gurmar, under no starvation condition almost 80–90% of flies fed on sugar (Figure 4A, C and E) compared to the starvation condition (24 h). We observed feeding responses were reduced to half ~30–50% in case of 10 and 5% gurmar fed flies after starvation (Figure 4B, D and F). Male flies specifically showed lower feeding (~5–10%) on 50 mM sucrose compared to female flies (Figure 4G and H) when males and females were tested separately after 24 h starvation. Under the no starvation condition, feeding responses of males and females were not significantly different (Figure 4G and H). We found no immediate effect of gurmar on sugar intake, in fact, we saw enhanced feeding on sugar in a fed state. Our data showed reduced sugar consumption only after starvation (24 h). Altogether our results suggest that the effect of gurmar is internal state dependent and sexually dimorphic as observed for reduced sugar intake in males after 24hrs of starvation.

A similar reduction in sugar consumption under starvation was also observed for many other sugars, namely 100 mM sucrose, fructose, trehalose, D-glucose, L-glucose, galactose, arabinose and sorbitol, except sucralose, L-glucose and galactose (Figure 5A). We also tested other taste categories including 50 and 200 mM NaCl and found no difference in feeding between normal media, 5 and 10% gurmar media condition (Figure 5B). Feeding responses for bitter compounds 10 mM caffeine, 1 mM denatonium and 1 mM quinine were no different between normal media, 5 and 10% gurmar media condition (Figure 5C). Various acids tested at 1% concentration also showed no difference in feeding (Figure 5D).

As a whole, our results suggest that gurmar modulates only sugar taste behaviour (although not all sugars) and the phenotype is specific for sugars. In general, wild type flies feeding on low-calorie sweeteners including sucralose, L-glucose and galactose showed lower feeding responses (<50%) under all conditions. Our model supports the mechanism where gurmar affects sugar intake in two different internal states (fed and starved, Figure 6). Under the fed condition, flies pre-fed on gurmar consume a high amount of sugar compared to normal media hungry flies (starved 24hrs) (Figure 6). Only after 24hrs of starvation time, flies pre-fed with gurmar showed reduced feeding suggesting a modulatory effect of gurmar on sweet taste behaviour. Where (brain or periphery) gurmar acts to cause the sweet taste modulation invites further probing. There is a possibility that taste memories under different internal states caused feeding differences in fed and starved flies. Also, the role of gurmar in altering the neuropeptides regulating feeding behaviour and its effect on the satiety region of the brain cannot be ignored which needs future investigation.

Discussion

Taste preference is essential for the evaluation of food by the animals. Sweet compounds provide energy to nearly all animals however, consumption of sugar should be regulated to maintain the proper body weight and overall fitness. People suffering from diabetes use different treatments to maintain their body weight and lower sugar levels. Our study used gurmar, which is in use for suppressing the craving for sugars and for balancing sugar levels in the body. Although, *G. sylvestre* use to control diabetes is well known in traditional systems of medicine and several studies have been conducted to report the antidiabetic properties and efficacy of *G. sylvestre*,^{31–34} but the scientific validation to establish the fact by conducting clinical trials with diabetic patients³¹ is lacking. Clinical trials done so far have used either crude extracts or whole leaf powder supporting the antidiabetic properties of *G. sylvestre* either by increased hypoglycemic activity³⁵ or enhanced insulin levels to a certain extent.³¹ Without knowing the exact mechanism and their after effects, the product is in use in raw powdered form (dry leaf powder). Even though various studies have suggested different doses for the treatment, the dose which is safe for human consumption is not clearly defined. Our study supports the role of gurmar in its raw powdered form in reducing partial sugar consumption in flies but only after a long starvation period (Figure 4 and 5). Our study presents evidences that flies perceive gurmar as an aversive cue and detect gurmar using both taste and olfactory systems (Figure 1 and 2).

Some clinical trials have been conducted using a water soluble extract of the leaves of *G. sylvestre* at a dosage of 400 mg/day in patients.³⁶ Absence of antidiabetic and hypolipidemic effects of *G. sylvestre* in non-diabetic and alloxan-diabetic rats have also been reported.³⁷ The rats fed with *G. sylvestre* leaf powder in the diet for 10 days prior and 15 days after the treatment of beryllium nitrate significantly protect the animals from the complete fall of blood glucose.³⁸ Our data suggest that flies pre-fed with gurmar consume less sugar only after at least 24 h of starvation time (Figure 4 and 5). We didn't observe any immediate effect of gurmar in altering the sugar feeding behaviour, in fact, flies with no starvation flies consumed more sugar compared to control wildtype flies on normal media (Figure 4). Our study presents results suggesting that although gurmar (in its dry leaf powdered form) has modulatory effects on sweet taste behaviour, but it takes much longer to show a reduction in sugar intake (Figure 4 and 5). So far no other study has presented a clear time scale where gurmar suppresses sugar consumption partially or completely.

Pharmacological studies suggest that the water extracts of *G. sylvestre* leaves effectively treat diabetes mellitus.³⁹ Similarly in rats, water extract of *G. sylvestre* leaves inhibit the absorption of glucose in the small intestine and suppress the increase of blood glucose value after administration of sucrose.⁴⁰ So far no study has highlighted the effect of gurmar under different internal states (fasting verses non-fasting) on the consumption of sugar in any animal model. Moreover, our results suggest that whole gurmar (dry leaf powder) consumption has deleterious effects on the development and overall health of the organism as seen here in the case of *Drosophila* (Figure 3C). Our data suggest that one has to be cautious when considering such ways of treating diabetes or to control the body weight.

The effect of *G. sylvestre* in alloxan induced diabetic rabbits by feeding crude leaf powder at a dosage of 250 mg/kg body weight once a day (did not feed animals every time before the meal) produced blood glucose homeostasis and increased activities of enzymes affording the utilization of glucose by insulin dependent pathways. The inhibitory effects of *G. sylvestre* and purified gymnemic acid on Gastric Inhibitory Peptide (GIP) release were studied in rats. The inhibition of GIP release by gymnemic acid was attributed to the interaction with the glucose receptor and found similar in specificity to the active glucose transport system. These results suggested that a glucose receptor interacts with the leaf extracts of *G. sylvestre* and purified gymnemic acid.⁴¹ We also found a reduction in sugar intake for many sugars after a long starvation time in our invertebrate model system (Figure 5A). Our studies do not claim where and how gurmar interacts to modulate the sweet taste behaviour. The identity of specific sugar receptor or neurons that interacts with gurmar is yet to be found. Our data suggest that gurmar might possibly interact with many sugar receptors in flies to modulate sugar consumption depending on the internal state of the animal and may have effects on higher brain centres (memory) as well (Figure 6).

Modern dietary supplements containing *G. sylvestre*, taken by diabetic patients, are typically intended to control sugar cravings and help with weight loss.³⁶ Hence, many pieces of evidence have supported the fact that gurmar reduces the blood sugar level. Our study focused for the first time on how whole gurmar (in raw dry leaves powdered form) acts on sweet taste feeding behaviour and alters the sugar intake in insects. For the same, we have compared our results in the healthy wildtype flies without using any obese or mutant flies that suffer from high levels of circulating sugar in the body. Our data on gurmar suggests that apart from its modulatory effects on sugar intake after starvation, it has detrimental effects on the health of the flies if fed throughout (Figure 3). Also, flies lay a fewer number of eggs on gurmar media and die earlier (Figure 3B), suggesting its potential in developing cost-effective strategies for pest control using the raw powdered form of gurmar.

How gurmar blocks the sugar receptors and acts on taste circuits or higher brain centres to modulate the sugar feeding is not known (Figure 6). Detailed examination of the impact of gurmar molecularly and behaviourally on appetitive cues like sugar on the brain's reward circuitry that leads to overeating and metabolic issues will further help in understanding underlying mechanisms that drive changes in neural activity. Understanding how gurmar reshapes sugar taste curves to promote reduced consumption of carbohydrates and sugars in flies may open up avenues to help people with health issues related to high sugar consumption by reducing sugar intake to the recommended level. Our study is an advancement in the understanding of how gurmar intake modulates sweet taste behaviour using *Drosophila* as a model system. This is the first kind of study that shows modulation of sweet taste behaviour in two different internal states (fed and starved) after pre-consumption of gurmar in flies. We do not intend to claim that these results support or are in contradiction of gurmar usage by humans.

Experimental procedures

Fly stocks

CsBz wildtype flies were received from National Centre for Biological Sciences (NCBS-TIFR), Bangalore, India. *Drosophila* were reared on standard cornmeal dextrose medium at 25° C, unless specified otherwise.

The media composition for *Drosophila* used is (1 liter of media) - corn flour (80 g), D-glucose (20 g, SRL-CAS Number-50-99-7), Sugar (40 g), Agar (8 g, SRL-CAS Number-9002-18-0), Yeast powder [15 g, SRL-REF-34266 (YI 012)], propionic acid (4 ml, SRL, CAS Number-79-09-4), TEGO (1.25 g in 3 ml of ethanol, fisher scientific, CAS Number-99-76-3), and Orthophosphoric acid (600ul, SRL, CAS Number-7664-38-2).

Feeding assays

For binary choice feeding assays, wildtype flies were raised from eggs to adults at 25° C. Flies were sorted in vials of 10 males and 10 females (20 flies/vial) upon eclosion and maintained at 25° C for 3 days on fresh media, after which flies were starved for 24 h at 25° C. Flies were tested as described previously,^{42,43} and abdominal coloration was scored as positive if there was any pink or red eating. Purple scored as 1/2 (when consumed red and blue dyes both) and none (no visible pink or red coloration). 60 × 15 mm feeding plates from Tarsons were used for the assay.

% flies feeding was calculated as follows: First, we calculated % flies feeding on gurrmar for each plate. Later mean of % flies feeding for 6 plates was calculated.

Tarsal proboscis extension reflex (PER) assay

2–3 days old wildtype flies were collected after eclosion and kept on standard food for 3 days. Mix of both male and female flies were used for the PER assay. Flies were tested as described previously,⁴³ Before the assay, flies were starved for 24 h in vials with water-saturated (4 ml) tissue papers. Prior to the PER experiment, no chemical anesthesia was used, instead flies were immobilized by cooling on ice for at least 20 min and then mounted using nail polish, vertical aspect up, on glass slides (76 mm × 26 mm × 1 mm from Borosil). Mounted flies were allowed to recover in a moist chamber (plastic box with wet tissues) for at least 1–2 h prior to testing. Tastant solutions prepared in water were applied directly to tarsi via a drop extruded using 2- μ l pipette. Flies were allowed to drink water *ad libitum* before testing the compounds. Flies not responding to water were excluded before the assay. Flies showing no movement were also excluded. Flies satiated with water were then tested with sucrose and gurrmar (whole gurrmar with liquid and solid particles floating was taken immediately after vortexing). Ingestion of any tastant solutions was not allowed and following each tastant application, flies were retested with water as a negative control. Each fly was tested five times with tastant solution stimuli (tastant was directly applied to the tarsi). The interval between consecutive tastant solution applications was at least 2–3 min to minimize adaptation. Flies showing three or more proboscis extensions were considered responders.

For all PER experiments, three sets of at least 20 flies each were tested and the percentage of responders was calculated for each set. PER graphs depict mean responses and error bars indicate standard error of the mean (SEM).

Chemicals used

All the sugars used in the study were obtained from Sigma Aldrich- Sucrose (57-50-1), Fructose (57-48-7), Trehalose (6138-23-4), Sucralose, D-(+)-Glucose (50-99-7), L-(-)-Glucose (921-60-8), Galactose (59-23-4), Arabinose (10323-20-3), Sorbitol (50-70-4). NaCl salt (fisher Scientific- 7647-14-5) was of 99.9% purity. Gurmar (Neutraved Gurmar powder from Amazon), Blue dye- Indigo carmine (Sigma: 860-22-0) and Red dye-Sulforhodamine B (Sigma- 3520-42-1). Bitter and acid compounds used were also obtained from Sigma Aldrich- Caffeine (58-08-2), Denetonium Benzoate (3734-33-6), Quinine (130-95-0), Linolenic Acid (60-33-3), Octanoic Acid (124-07-2), Hexanoic Acid (142-62-1), Acetic Acid (64-19-7), Citric Acid (5949-29-1), Glycolic Acid (79-14-1).

Spectrophotometry analysis

After 2 h of feeding on blue spots with the tastant (50 mM sucrose, 2.5% gurmar, 5% gurmar, 10% gurmar and various concentrations of gurmar mixed with sucrose) flies were pooled in equal numbers (60 flies \times 2 sets) for each condition and were put in 2 ml eppendorf in 70% ethanol (Figure 1C). Flies were first crushed in 150ul of 70% ethanol and then 150ul 70% ethanol was added to crush them more. After crushing, 200 ul of double-distilled water (to remove the content sticking to the pestle and wall of the eppendorf tube) was added in the same soup and centrifugation was done at 3000 rpm for 15 min. After centrifugation, the pellet was discarded and the supernatant was transferred into the fresh eppendorfs. To do the spectrophotometry analysis, the supernatant was further diluted with 350 ul double distilled water to make up the total final volume of 600ul in the cuvette. Spectrophotometry analysis was done at 630 nm wavelength. Readings were taken for each sample and only the mean values were plotted. The spectrophotometer used was Perkin Elmer, lambda 35 UV/VIS Spectrometer.

Lethality profile

To calculate the number of flies dying on 5 and 10% gurmar mixed with fly media, for each concentration of gurmar 20 flies in each vial (10 male and 10 females) were kept in batches (X6). Flies were transferred on alternate days to fresh media and the number of flies dying every day were counted for the days specified in the graph. Flies kept on normal media were used as the control.

Egg counting

Mated flies were kept on normal media first to synchronize the flies and then shifted to different media conditions (NM-normal media, 5% gurmar mixed with normal fly media and 10% gurmar mixed with normal fly media) for egg laying. Synchronization was done for two days by transferring flies on normal media for each condition at least three times a day. Each vial had 25 females and 15 males (N = 6 vials for each condition). Flies were transferred on different media plates for egg collection. Yeast paste was used to stimulate the

mating. After 20 h, flies were removed from the egg chamber plates (NM-normal media, 5% gurmar mixed with normal fly media and 10% gurmar mixed with normal fly media). After egg laying, the number of eggs was counted for each plate and mean number of eggs was calculated and plotted for each condition.

Developmental profile analysis

Synchronized flies were transferred to fresh normal media vials, 5% gurmar mixed in normal media vials and 10% gurmar mixed in normal media vials. Flies were allowed to lay eggs for two days and later removed from the vials. Fly development, eclosion time and total flies emerged were observed for the next 7 days. N = 6 vials (20 flies each).

Fly size measurement

While measuring the length of the flies only the body was considered. Wings were not considered to measure body size.

Microscopy used for image analysis and movie making

Olympus SZX10 dual tube microscope was used for generating images and Olympus SZ61 stereomicroscopes for doing the general fly pushing.

Statistical analyses

Unless otherwise stated, differences between means of different groups were evaluated for statistical significance with parametric ANOVA followed by post hoc Tukey multiple comparisons test for obtaining the p- values. For any other statistical analysis as well, only ANOVA was used.

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Author biographies

Shivam Kaushik is currently working at Regional Centre for Biotechnology, Faridabad, India, in Dr. Pinky Kain's Lab as a Lab technician. He is interested in understanding the functional role of neuronal circuits involved in memory formation in the brain to develop therapeutic strategies and tools for diagnosing and treating neurodegenerative disorders like dementia.

Rahul Kumar is a PhD student at the University of New Mexico. He is involved in conducting and interpreting experiments on mouse models of heavy metal exposure. He is focused on understanding the role of heavy metal exposures and key drivers of hepatobiliary cancer disparities seen commonly in New Mexico. The experiments involve studies focused

on cell viability, protein expression and metabolomics of liver and gallbladder tissues in mouse models.

Sachin Kumar is currently working on renewable energy. He is working in his own company called Viresco Technologies LLP, Roorkee, in India.

Srishti Sanghi is currently a PhD student at the Indian Institute of Technology, Jodhpur, India, whose research interest includes understanding the intricacies of cancer and how centrosomes and associated proteins aid in modulating the severity of the disease.

Pinky Kain is currently working at Regional Centre for Biotechnology, Faridabad, India as a Wellcome Trust DBT India Alliance Fellow. She runs her own laboratory of Neurobiology and Genetics where her group studies peripheral and central taste coding and modulation using *Drosophila melanogaster*.

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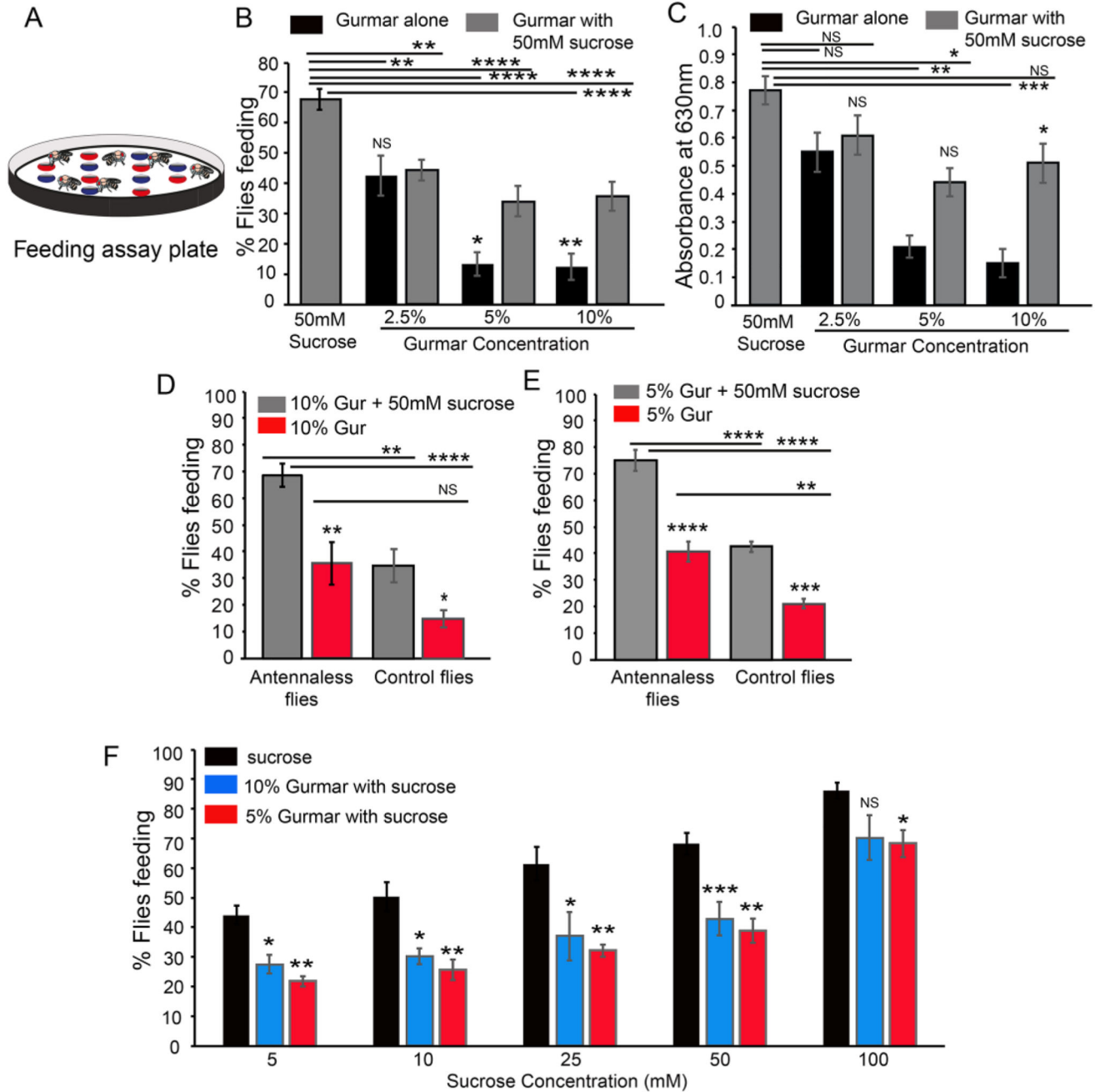


Figure 1. Feeding behaviour of wildtype flies on gurmar.

(A). Cartoon showing feeding assay plate. Feeding assay plate displaying red spots containing gurmar, agar and water. Blue dots represent control dots containing water and agar with the dye. In our two-hour feeding assay, flies could choose between water and varying concentrations of gurmar in an unbiased manner in the dark. All the experiments were conducted at 25° C. Gurmar alone or mixed with sucrose in agar and water was presented as red spots (Figure 1A and B). Sucrose (50 mM) was treated as a positive control. Flies (%) that were feeding on gurmar were scored based on abdomen color (white- no

feeding, blue-feeding on agar and water, red or pink-feeding on gurmar, purple-feeding on both red and blue). (B) Graph shows the mean feeding responses of wildtype *CsBz* flies for various concentration of gurmar in the feeding assay. The gray bar represents feeding data on gurmar when mixed with 50 mM sucrose and black bars represent feeding on only gurmar (2.5%, 5% and 10%). For each bar, n = 6 trails of ~20 flies each for each concentration (10 males and 10 females). 50 mM sucrose (First gray bar) was used as a positive control. (C) Spectrophotometry analysis of wild type *CsBz* flies for various concentrations of gurmar after the feeding assay. For each bar, n = 2 sets of 60 flies each for each tastant (each set- 30 male and 30 females). (D-E) Mean feeding responses of wildtype antennaless flies after feeding on gurmar (10% gurmar and 10% gur + 50 mM sucrose; 5% gurmar and 5% gur + 50 mM sucrose). Wildtype flies with intact antennae were used as controls. Before performing the experiment, antennaless flies were placed in normal media vials for two days to normalize. n = 6 trails of ~20 flies each for each condition (10 males and 10 females). (F) Mean feeding responses of wild type flies for 5 and 10% gurmar with increasing concentration of sucrose. For all the experiments, starvation time is 24 h. For each bar, n = 6-12 trails of ~20 flies each for each concentration (10 males and 10 females). For all the graphs, statistical analysis was performed using ANOVA Tukey's multiple comparison test for obtaining P values: *p < 0.05, **p < 0.005 and ***p < 0.0005 (ns, not significant), error bars = SEM.

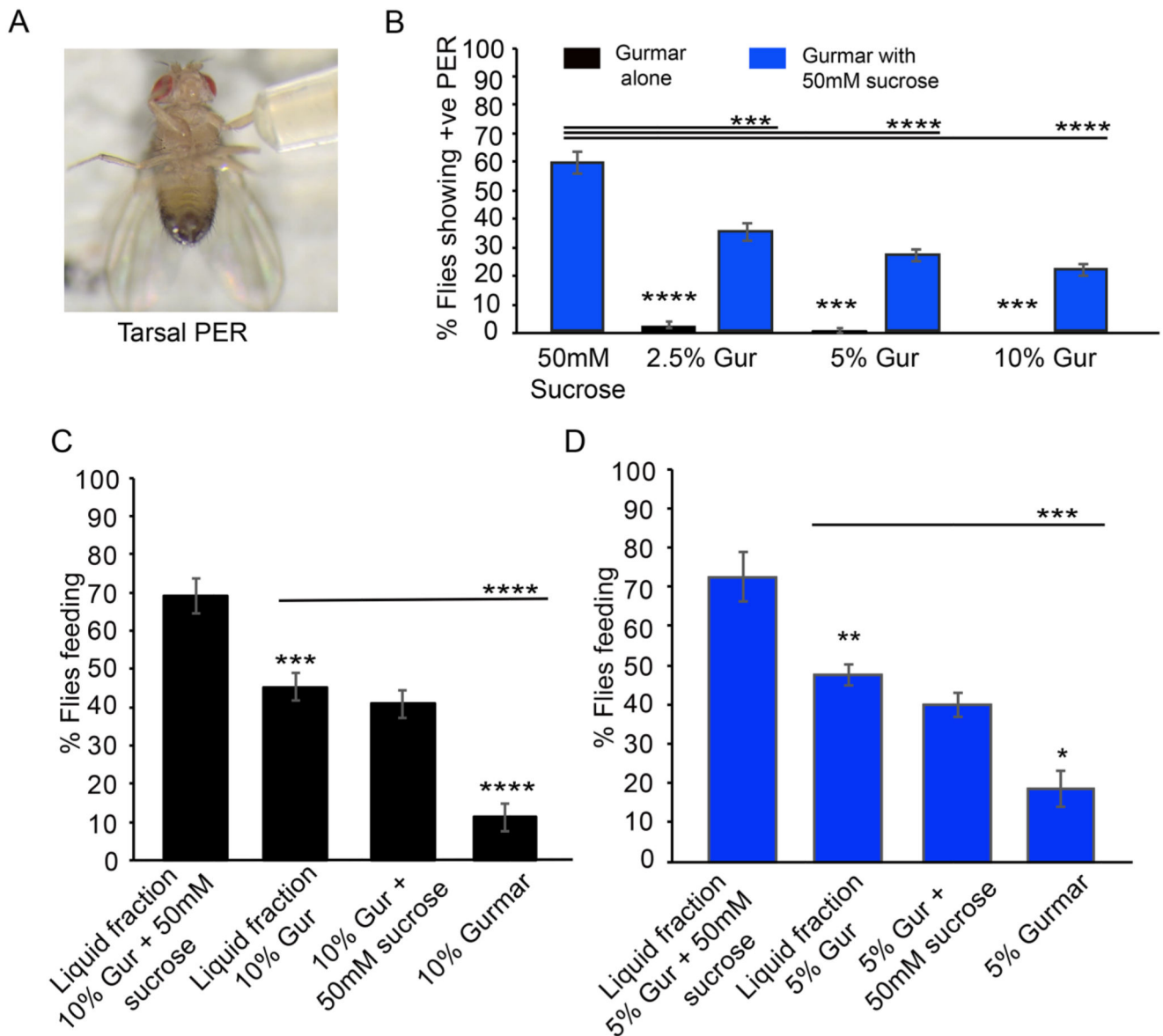


Figure 2. Whole gurmar is aversive to flies.

(A) Snapshot (bright field image) of tarsal proboscis extension reflex (PER) assay. (B) Graph showing mean PER responses of wildtype flies (male and females mixed) for 2.5%, 5% and 10% gurmar concentration alone (black bars) and 10% gurmar mixed 50 mM sucrose (blue bars). 50 mM sucrose served as a positive control (first blue bar). For each bar, $n = 100$ flies for each concentration (5 slides \times 20). Before testing gurmar, sucrose stimulus was given at the start and at the end of the experiment to check for any adaptation. Only flies showing + ve responses (in the beginning and at the end) were considered for the sucrose response. (C) Feeding response of flies after feeding on a liquid fraction of the 10% gurmar solution + 50 mM sucrose and liquid fraction of whole gurmar (10%). For these experiments, only the upper liquid fraction was used for testing in the feeding assay. 10% whole gurmar alone and 10% whole gurmar mixed with 50 mM sucrose in

feeding assay were also tested. For performing assays with whole gumar, the whole content of the solution (solid and liquid components) was used and mixed with agar and dye. For all the experiments, starvation time was kept as 24 h. For each bar, n = 12 trails of ~20 flies each for each concentration (10 males and 10 females). (D) Feeding response of flies after feeding on the liquid fraction of the 5% gumar solution + 50 mM sucrose and liquid fraction of whole gumar (5%). The experiment was done as stated for 10% gumar in C. For each bar, n = 6 trails of ~20 flies each for each concentration (10 males and 10 females). Statistical analysis was performed using ANOVA Tukey's multiple comparison test for obtaining P values: *p < 0.05, **p < 0.005 and ***p < 0.0005 (ns, not significant). Error bars = SEM.

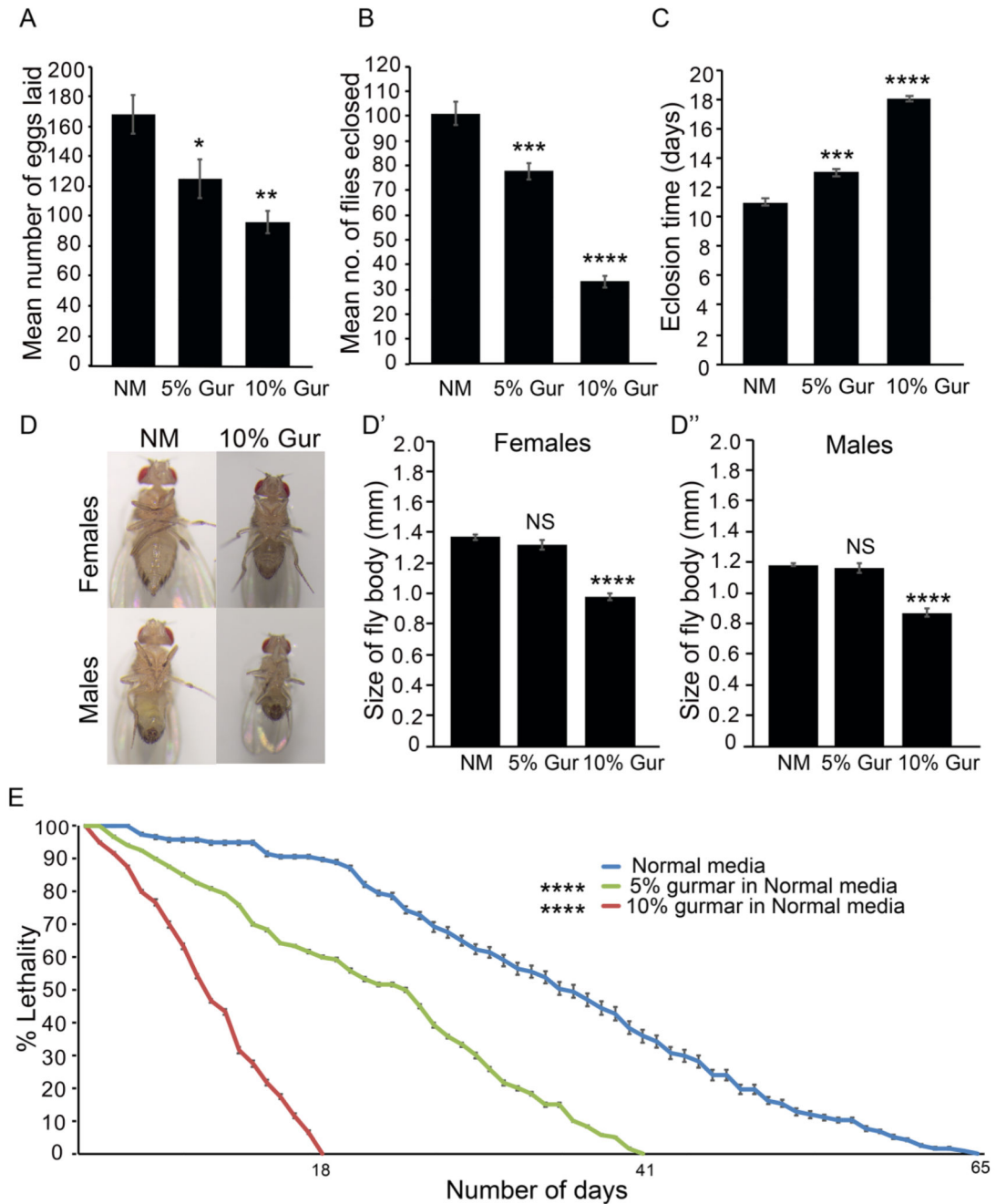


Figure 3. Effect of gurmar feeding on egg laying behaviour and development of flies.

(A) Egg laying behaviour of flies on gurmar and normal media (N = 6 plates in each case) plates. 5 and 10% gurmar was mixed with standard fly food. (B) Graph showing the mean number of flies eclosed (N = 6 vials in each case). (C) Graph showing eclosion time (N = 6 vials in each case) for flies on normal media (NM), 5% and 10% gurmar vials. (D) Images of flies showing developmental defects when grown on 10% gurmar mixed with normal media compared to wild type flies grown on normal media (NM). Flies were maintained at 25°C throughout. (D' and D'') Mean body size of male and females flies after eclosion on 5

and 10% gurmar mixed with normal media (N = 6 flies in each case). (E) Life span analysis of wild type flies feeding on normal media (blue line), 5% gurmar mixed with normal media (green line) and 10% gurmar mixed with normal media (red line). N = 6 vials each condition, each vial contained 20 flies-10 males and 10 females. For all graphs, statistical analysis was performed using ANOVA Tukey's multiple comparison test for obtaining P values: *p < 0.05, **p < 0.005 and ***p < 0.0005 (ns, not significant). Error bars = SEM.

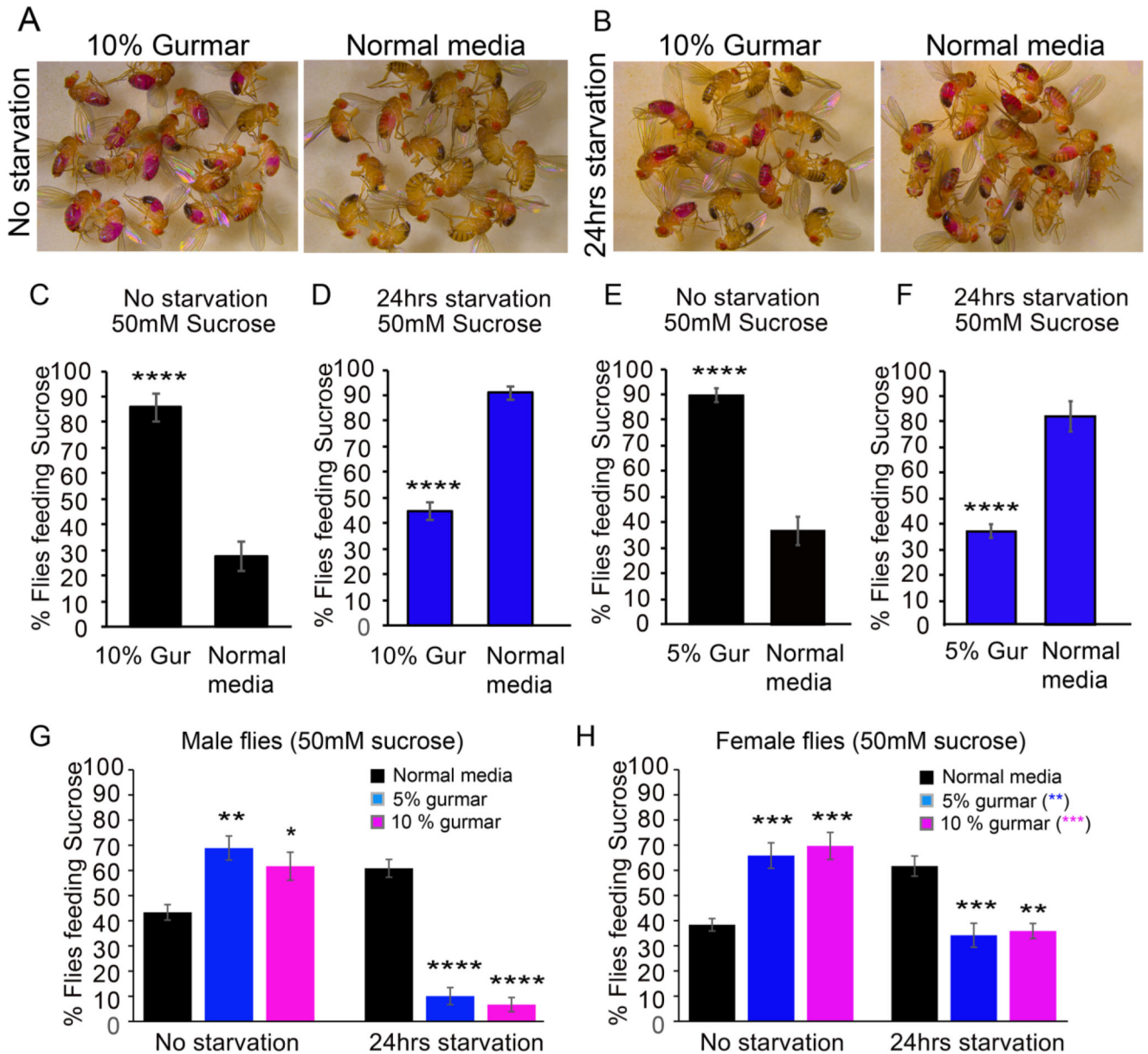


Figure 4. Feeding on gurmar affects the sugar intake only after starvation.

(A) Images of flies after feeding 2 days on 10% gurmar mixed with normal media and tested for 50 mM sucrose in the feeding assay. Normal media-fed flies treated as controls. (C and E) Graph showing consumption of sucrose in flies after no starvation for flies fed on 10 and 5% gurmar mixed with normal media. (D and F) Graph showing consumption of sucrose after 24hrs starvation for flies fed on 5 and 10% gurmar mixed with normal media. (G) Feeding response of male flies under no starvation and 24 h starvation condition. (H) Feeding response of female flies after no starvation and 24hrs starvation condition. The difference between male and female flies at 5 and 10% gurmar for 24hrs starvation condition is shown in H as blue and magenta asterisks. For all the experiments, starvation time was 24 h. For each bar, n = 6 trails of ~20 flies each for each concentration (10 males and 10

females). Statistical analysis was performed using ANOVA Tukey's multiple comparison test for obtaining P values: * $p < 0.05$, ** $p < 0.005$ and *** $p < 0.0005$, (ns, not significant).

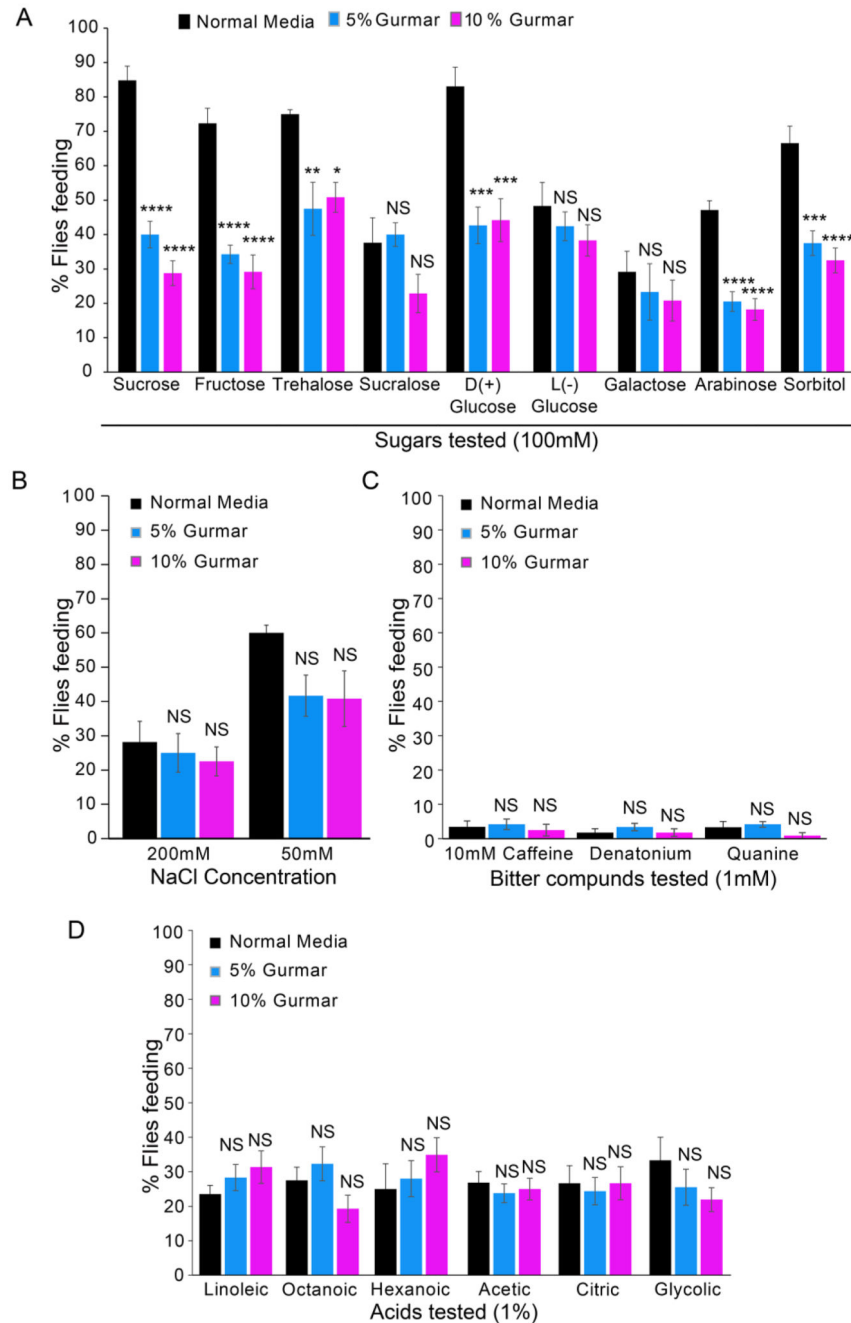


Figure 5. Feeding on gurmar affects only sugar intake after starvation.

(A) Mean feeding responses of wild type flies when kept on 2 days on 5% and 10% gurmar mixed in normal media and tested for different sugars. Graph showing feeding responses for different sugars tested at 100 mM concentration (Sucrose, Fructose, Trehalose, Sucralose, D (+)-glucose, L (-) - glucose, Galactose, Arabinose and Sorbitol). (B) Graph showing feeding responses for 200 mM and 50 mM NaCl. (C) Graph showing feeding responses for different bitter compounds tested (10mM Caffeine, 1 mM Denatonium and 1 mM Quinine). (D) Graph showing feeding responses for different acids tested (Linoleic acid, Octanoic

acid, Hexanoic acid, Acetic acid, Citric acid and Glycolic acid) at 1% concentration. For all the experiments, starvation time is 24 h. For each bar, n = 6 trails of ~20 flies each for each concentration (10 males and 10 females). Statistical analysis was performed using ANOVA Tukey's multiple comparison test for obtaining P values: *p < 0.05, **p < 0.005 and ***p < 0.0005. For all graphs, error bars = SEM.

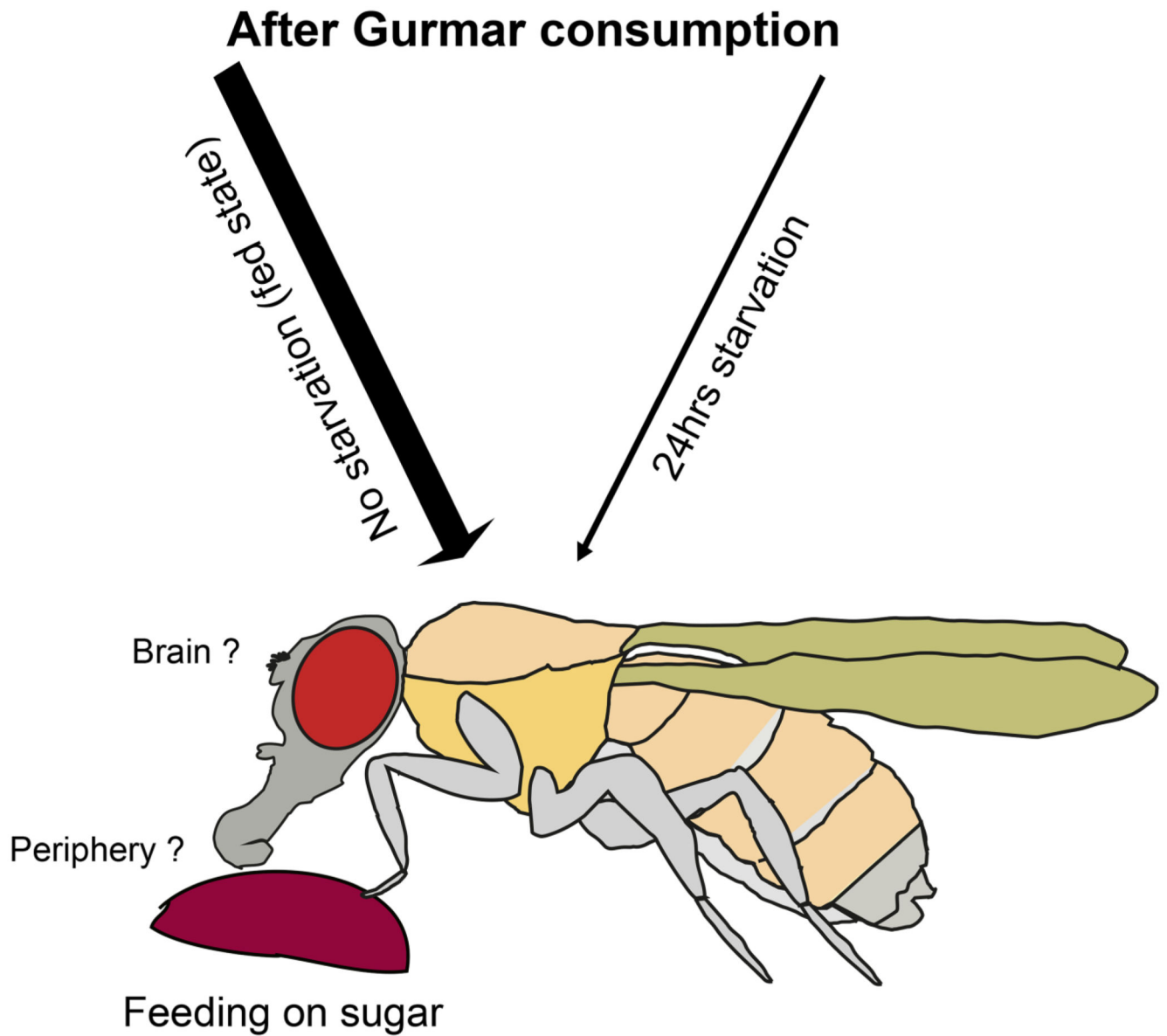


Figure 6.

Model-Pre-consumption of gurmar for 2 days in flies under the fed state cause increased consumption of sugars. On the other hand, a long starvation time (24 h) causes a reduction in sugar intake. The thickness of the arrows is indicative of the strength of sugar consumption. Where gurmar acts (periphery, central neuronal circuits, taste cells, satiety centres and memory centres) to cause feeding modulation is still undetermined and needs further investigation.