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Research Article

EFFECTS OF NON-NUTRITIVE SWEETENERS ON TOXICITY AND MATING BEHAVIOUR IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The intake of sweeteners and sweetener containing foods has been increasing day by day, leading to the development of certain health problems, survival, and reproduction. Over the past few years, there has been an enormous growth in the study of sweetener substitutes as dietary assessments help in understanding the nutritional intake levels; however, its ill-effects on life history traits have not been studied. In the present study, the effect of two Non-Nutritive Sweeteners (NNS), Aspartame and Sucralose and nutritive sweetener, Sucrose are been studied on toxicity, climbing ability and mating behaviour using *Drosophila melanogaster*. Control flies had the least mortality rate whereas the highest mortality rate was noticed in aspartame treated flies. Based on the mortality, LD_{50} values were fixed for NNS for further experiments. Five days old virgin females and unmated males obtained from control and LD_{50} treated media (Aspartame/Sucralose/Sucrose) were used for climbing ability and mating behaviour experiment. Control flies had a significantly greater climbing ability than nutritive and NNS treated flies. Further, NNS treated flies showed the least climbing ability. The highest mating success was noticed in control flies and the least mating success was found in flies treated with NNS. Aspartame treated flies took a long time for mating whereas control flies had taken the least time for mating. Further, male courtship activities were least in flies treated with NNS whereas control flies showed the highest courtship activities. On the other hand, female rejection responses were highest in flies fed with NNS and least among control flies. Thus, these studies in *Drosophila melanogaster* suggest that NNS such as aspartame and sucralose have toxic effects and have negative effects on fitness such as climbing ability, mating behaviour, and mating success.

Keywords: *Drosophila melanogaster*, Non-Nutritive Sweeteners (NNS), Climbing ability, Mating behaviour.

1. INTRODUCTION

The intake of sweeteners and sweetener containing foods has been increasing day by day which has led to the development of certain health problems. Obesity, diabetes, cardiovascular disease cases are prominent because of an increase in blood sweetener levels which further cause detrimental effects [1]. Management in controlling the disease among the population is very much needed. Therefore, low calorie or no calorie sweeteners can be restored with nutritive sweetener. Over the past few years, there is an enormous growth in the study of sweetener substitutes as dietary assessment help in the nutritional intake levels [2]. These sweetener substitutes called as Non-Nutritive Sweeteners (NNS) or artificial sweeteners produce sweetening effects without adding carbohydrates or calories to the food [3]. Today, food industries have come up with several different forms of NNS in the management and control of obesity, diabetes, and heart diseases throughout the world [4].

Aspartame and Sucralose are the synthetic NNS most widely used as sweeteners and also approved in many types of products. Aspartame is 200 times sweeter than sucrose whereas Sucralose is 600 times sweeter than sucrose with no calories [5]. Studies using these sweeteners instead of normal sweeteners may have favorable effects on bodyweight, blood glucose level, insulin resistance, no elevation in craving behavior, less fat accumulation and lower lipogenic effects [6]. In contrast, this information of experiments on rats is presented by the adverse effects of aspartame have been shown where lifetime feeding of aspartame on rats increased risk for lymphoma, leukemia and transitional cell carcinoma of pelvis, ureter, bladder in a dose-dependent manner [7]. A further recent study on mice concerning NNS believes that long term consumption of aspartame leads to the imbalance in antioxidant/

peroxide status in the brain of rats exposed to100mg/kg body weight [8]. An interesting study by Brown *et al* [9] on a large number of volunteers showed increased waist circumference after consuming diet coke containing NNS. Further, many studies have shown the effect of these NNS on the gut microbiota population and there is a reduction in the few types of microbiota or alteration in the gut microbiota upon consumption of aspartame [10].

Despite its use, Aspartame has been the most controversial NNS because of its potential toxicity [11]. On digestion, aspartame breaks down into amino acids aspartic acid and phenylalanine and small amounts of methanol. High intake of aspartame has been reported with symptoms like headaches, blurred vision, blurred numbness, insomnia, memory loss, loss of energy, hearing problems, behavioural disturbances, neuro-logical and carcinogenic effects [12, 13]. Sucralose is a sugar substitute that has a sweet flavour and sweetness intensity close to sucrose. Despite the fact that sucralose is produced from sucrose, the human body does not recognise it as sugar and metabolise it, therefore it has no calories. Many of the research were designed to find potential toxic effects, such as cancer, reproductive, and neurological impacts, and none have been identified. The FDA (Food and Drug Administration) has approved based on studies indicating sucralose is safe for human consumption [14].

The LD_{50} (Lethal Dose) is the amount of a substance which causes death of 50% (one half) of a group of laboratory animals when administered all at once. LD_{50} is one of the methods of determining a substance's poisoning risk at limited time. NNS can affect our health in a variety of ways. Toxicity testing in lower species may be necessary depending on how the NNS will be used. Hence, to compare the toxic potency or severity of different NNS on physical and mating behaviour, we used lethality testing (LD_{50} test) in *Drosophila melanogaster (D. melanogaster)* to determine how much quantity of millimolar (mM) is needed to cause 50% death in the given number of samples in each concentration of different NNS and NS.

However, the toxic effects of these NNS on the relationship of life span and reproductive success are yet to be studied. Therefore, using *D. melanogaster* as a present model organism provides a range of advantages which include easy and reasonable cultures in the laboratory as its life cycle is short, have many offspring and short generation times [15]. Hence, the objective of this study was undertaken to study the effects of NNS (Aspartame and Sucralose) on survival and mating success in *D. melanogaster*.

2. MATERIAL AND METHODS

2.1. Experimental stock

The experimental stock of *D. melanogaster* was obtained from *Drosophila* Stock Centre, Manasagangotri, Mysuru. The flies were raised in different cultured bottles containing wheat cream agar media (100 g of jaggery, 100 g of wheat powder, 8 g of Agar- Agar was boiled in 1000 ml of double-distilled water and 7.5 ml of propionic acid was added at last). Twenty flies (10 males and 10 females) were introduced into culture bottles and maintaining at the temperature of 22±1°C with the relative humidity of 70% in 12 hours dark, 12 hrs light cycle. To acclimate flies to laboratory conditions, this procedure was repeated for three generations. Delcour's procedure [16] was used in the fourth generation to collect eggs from these flies. Each culture bottle with normal and treated media (Sucrose/ Aspartame/Sucralose) contained 100 eggs seeded in it. The NNS aspartame and sucralose, nutritive sweetener sucrose were dissolved separately in media at the concentration of 20,40,60,80,100 and 120 millimolar (mM) respectively and these media were used to determine LD_{50} using mortality rate. The flies obtained from LD_{50} treated media were used to study climbing assay and mating activities experiments. Control flies were raised in wheat cream agar media.

2.2. Validation of food intake by dye method

Adult flies were placed in a vial containing media treated with NNS [2.5 percent (w/v) blue food dye] (FD & C Blue Dye no. 1). The flies were allowed to feed for 24 hours. The addition of brilliant blue to the treated media provided visual confirmation of the consumption by *D. melanogaster*.

2.3. Quantification of Food intake in Larvae using dye method

The feeding behaviour in ten second instar larvae obtained from normal and treated media was studied. Each larva was placed in a vial containing control, aspartame, sucrose and sucralose-based media that were treated with 2.5 percent (w/v) blue food dye (FD & C Blue Dye no. 1). For 15 minutes, the larvae were allowed to feed. They were then frozen in an Eppendorf tube.

2.4. Effect of Non-Nutritive Sweeteners on mortality rate to determine LD_{50}

Five days old *D. melanogaster* flies were used for the experiment. Each replicate consisting of a set of 30 flies (15 males and 15 females) were transferred separately to the control and treated media. The treated media obtained are NNS Aspartame/ Sucralose, nutritive sweetener Sucrose was dissolved separately in media at the concentration of 20,40,60, 80,100 and 120 millimolar (mM) and the mortality rate was recorded at an interval of 24,48,72,96,120,144, 168 and 192 hrs/concentration/treated sample. Flies were considered to be dead if they showed no response in their behaviour and movement with gentle touching them. A total of 5 replicates of both normal and treated /concentration media were conducted separately. Data obtained were subjected to One-Way ANOVA followed by Tukey's post hoc test. LD_{50} was determined for each of the treated media.

2.5. Effect of Non-Nutritive sweeteners on climbing assay

Ten (five days old) experimental flies obtained from control and LD_{50} treated media (Sucrose/Aspartame/ Sucralose) were subjected to climbing assay to record the climbing ability of flies. A 25cm hollow tube was used, with one end of the tube covered with a cap and the other end with a cotton plug. The tube was labelled with three height levels: 0-8cm, 8-16cm, and 16-24cm. Flies were inserted into an assay tube and gently tapped to the bottom of the tube. Flies were allowed to ascend the tube after the stop clock was started. The data was recorded according to the fly's capability of climbing at different heights within 60 seconds. A total of 5 replicates for each of the control and treated media were conducted separately. One-Way ANOVA analysis followed by Tukey's post hoc test was carried out on climbing assay data.

2.6. Effect of Non-Nutritive Sweeteners on mating activity and courtship behaviours of *D. melanogaster*

Five days old virgin females and unmated males obtained from control and LD_{50} treated media (Aspartame/ Sucralose/Sucrose) were used for the experiment. A separate experiment was performed for flies raised from each of the control and treated media. Fifty pair-wise matings were made to study courtship patterns and mating activities. Each pair's mating latency (the time between introducing the male and female into the mating chamber until the start of copulation) and copulation duration (the time between the start of copulation and the end of copulation) were recorded following the procedure of Hegde and Krishna [17]. We have quantified male courtship activities such as tapping, scissoring, vibration, licking, and circling, and also female courtship acts such as ignoring, extruding, and decamping in this experiment.

- Tapping: male foreleg motion will initiate courtship. The male strikes downward after partially extending and simultaneously elevating one or both forelegs, bringing the ventral surface of the tarsus into contact with the partner.
- Scissoring: a courting male will sometimes open and close both wings with a scissor-like action during the intervals between wing vibrations.
- Vibration: during wing movements, males expand one wing laterally from resting posture and then raise the wing(s) quickly up and down.
- Licking: a courtship male approaches the female closely from behind, extends his proboscis, and licks her genitalia.
- Circling: after posturing to the side or back of a non-receptive female, the male will circle the female, facing her. He turns around to face her and then retraces his steps back to the rear; at other times, he circles her completely.
- Ignoring: When a non-receptive female is courted, she will simply continue doing her activity and ignoring the male's actions.
- Extruding: The vaginal plates are squeezed together by non-receptive females, which contracts certain abdominal muscles while relaxing others.
- Decamping: non-receptive females frequently attempt to escape the courting male by running, jumping, or flying backward.

The behaviour of males and females are recorded simultaneously for 1 hour; the number of pairs mated were also recorded.

3. RESULTS

3.1. Food intake using Dye method

The food intake of adult *D. melanogaster* flies was confirmed using 2.5% (w/v) blue food dye (FD & C Blue dye No.1) in the media. This experiment with adult flies was done to obtain the visual confirmation of consumption of the liquid dye by *D. melanogaster* (Fig. 1).

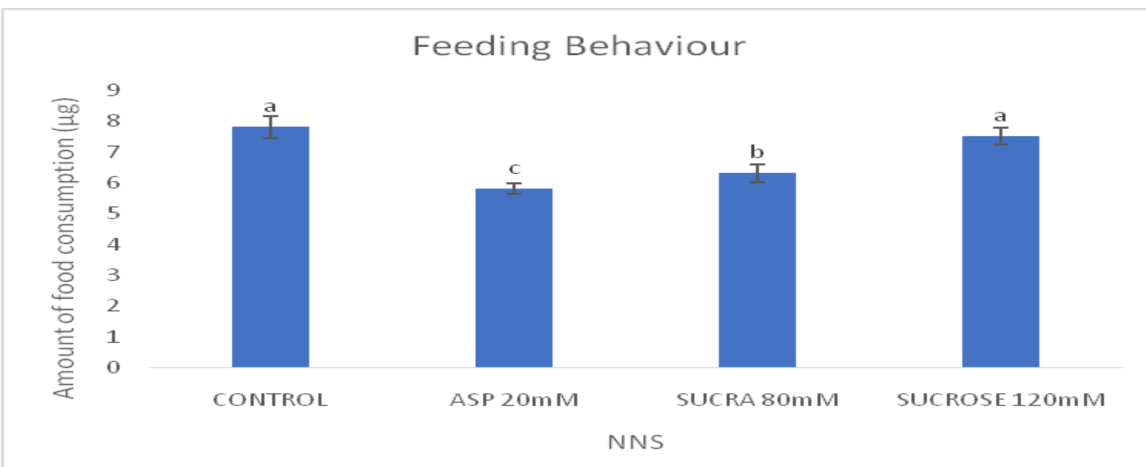
Fig. 1: Abdomen dye in male and female of *D. melanogaster*

3.2. Food consumption in larvae using Dye Method

Fig. 2 represents food consumption by *D.melanogaster* larvae using the dyeing process. When comparing NNS and control larvae, it was found that flies fed on sucrose treated media consumed more food. The data were subjected to SPSS and performed with One-Way ANOVA followed by a Tukey's Post Hoc test. Tukey's Post Hoc Test revealed a substantial variation in feeding rate among sugar-based media.

3.3. Mortality rate of *Drosophila melanogaster* in treated and control media.

The mortality of flies provides evidence of the dosage toxicity fed by the flies in the media. The mean mortality rate of control flies was less compared to treated media (Aspartame/Sucralose/Sucrose). Further in all the media used, mortality rate increased in the treated media. The mortality rate of sucrose treated flies were less compared to Aspartame or Sucralose treated flies suggesting that the mortality rate of flies was less in nutritive sweeteners than those of NNS. The mortality rate data subjected to One-Way ANOVA followed by Tukey's post-hoc test (Fig3) revealed that the mortality rate varied significantly between control and treated media and Tukey's post hoc test showed that among the treated media, mortality rate was significantly less compared to that of higher concentration's compound used in each of the three treated media. Based on the mortality, LD_{50} values were fixed as follows: 20mM for Aspartame, 80mM for Sucralose and 120mM for Sucrose for further experiments.

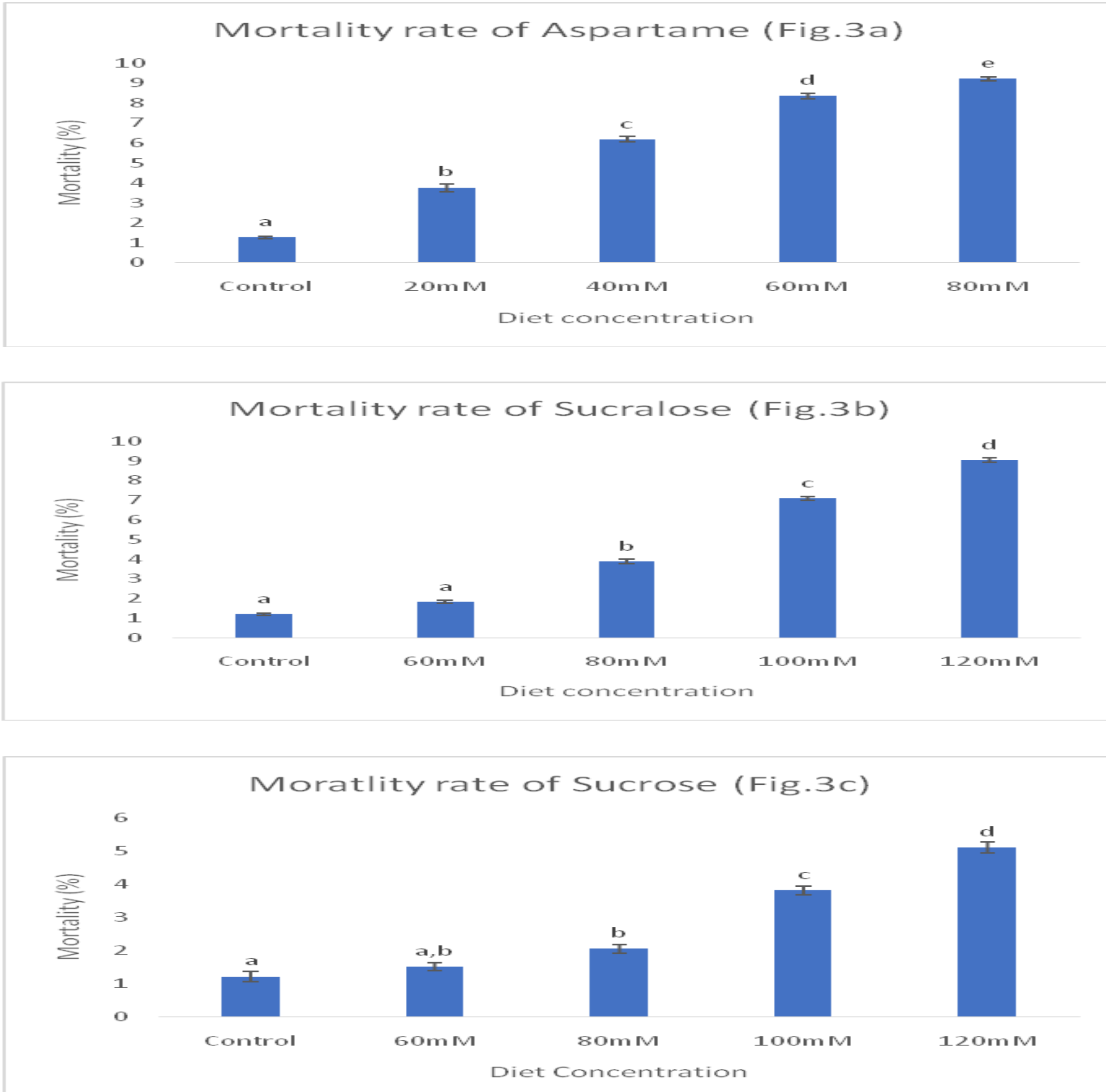


(Different letters on the bar graph indicate significance at 0.05 level by Tukey's post-hoc test)

Fig. 2: Feeding behavior of *D. melanogaster* in control and treated media

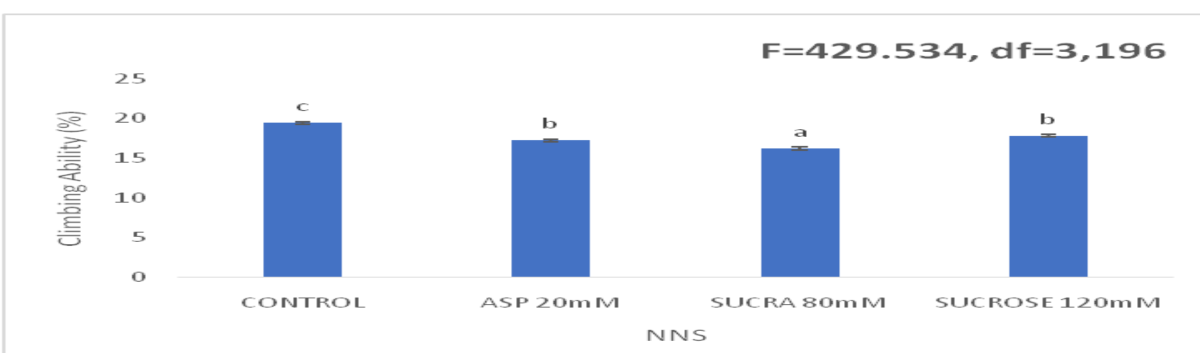
3.4. Climbing assay

The climbing ability result of the control and treated flies showed a significant difference in the climbing ability. The highest climbing ability was found in control flies whereas the least climbing ability was seen in sucralose treated flies. Further, nutritive sweetener, Sucrose treated flies were more capable of climbing than NNS treated flies such as aspartame and sucralose (Fig. 4). The Climbing ability data was subjected to One-way ANOVA followed by Tukey's post-hoc test. Tukey's post-hoc test revealed that climbing ability varied significantly between control and treated media.



Different letters on the bar graph suggest significance by Tukey's post-hoc test at the 0.05 level

Fig. 3: (a+b+c): Mortality rates of *D. melanogaster* in control and treated media (Aspartame/Sucralose/Sucrose)



Different letters on the bar graph suggest significance by Tukey's post-hoc test at the 0.05 level

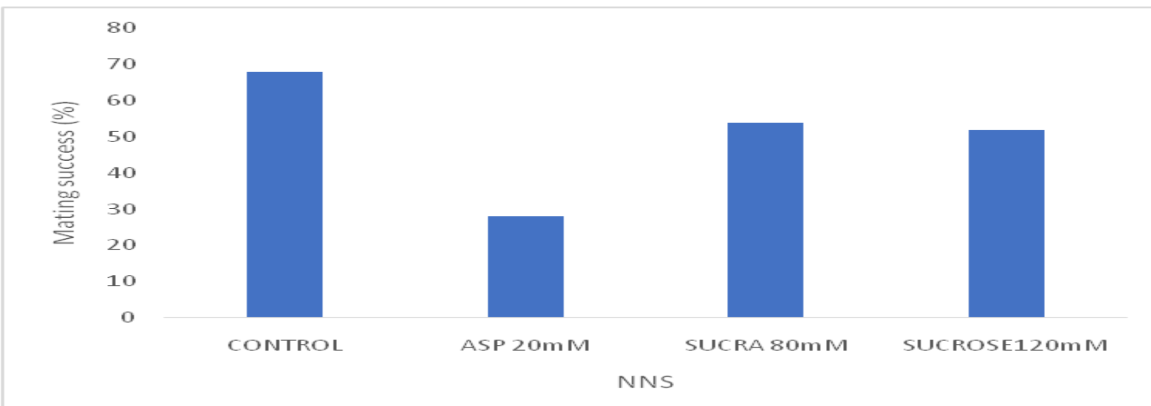
Fig. 4: Climbing ability of *D. melanogaster* in control and LD_{50} treated media (Aspartame/Sucralose/Sucrose)

3.5. Mating behavior

The percentage of mating success was highest in control flies and least in aspartame treated flies. Further, mating success was greater in nutritive sweetener treated flies compared to NNS treated flies (Fig 5). One-Way ANOVA followed by Tukey's post hoc test revealed that the percentage of mating success of flies differed significantly between control and treated media. Further even among the treated media, percentage of mating success was lowest in flies fed on aspartame treated media.

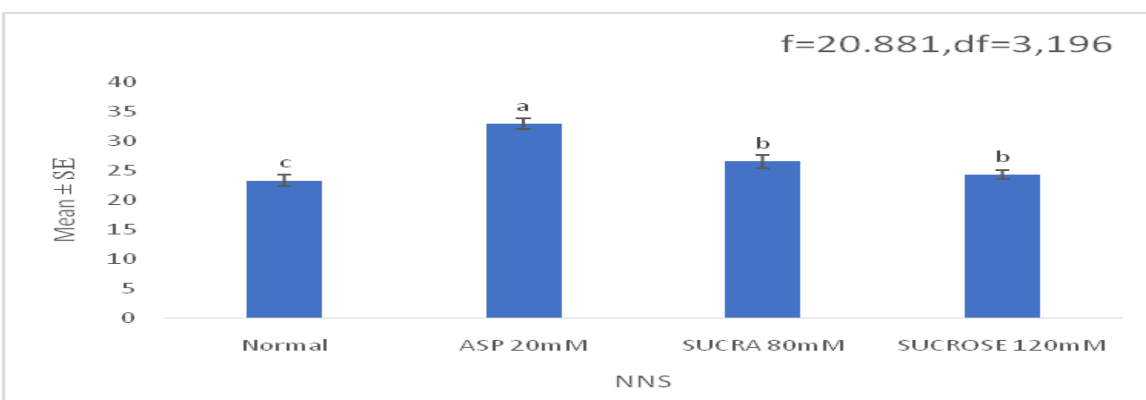
Courtship behaviour patterns varied significantly among the adult flies fed on different sweeteners. Mating latency was the longest in aspartame treated flies and the shortest in sucrose treated flies. Even among sweeteners used, flies fed with NNS had taken more time for mating than that flies fed with nutritive sweetener. One-Way ANOVA followed by Tukey's post hoc test revealed that mating latency varied significantly between different control and treated media flies. Further, Tukey's post hoc test showed that mating latency significantly varied between control and treated media flies and also between treated media flies.

Copulation duration was shortest in aspartame treated flies and longest in control flies. Among the treated flies sucrose treated flies copulated longer compared to flies treated with NNS aspartame and sucralose. Further, the least copulation duration was noticed in aspartame treated flies. One-way ANOVA followed by Tukey's post hoc test revealed that copulation duration varied significantly between control and treated media flies. Further, Tukey's post hoc test revealed that copulation duration differed substantially between control and treated media flies and also between treated media flies.



Different letters on the bar graph suggest significance by Tukey's post-hoc test at the 0.05 level

Fig. 5: Mating success of *D. melanogaster* in control and LD_{50} treated media (Aspartame/Sucralose/ Sucrose)

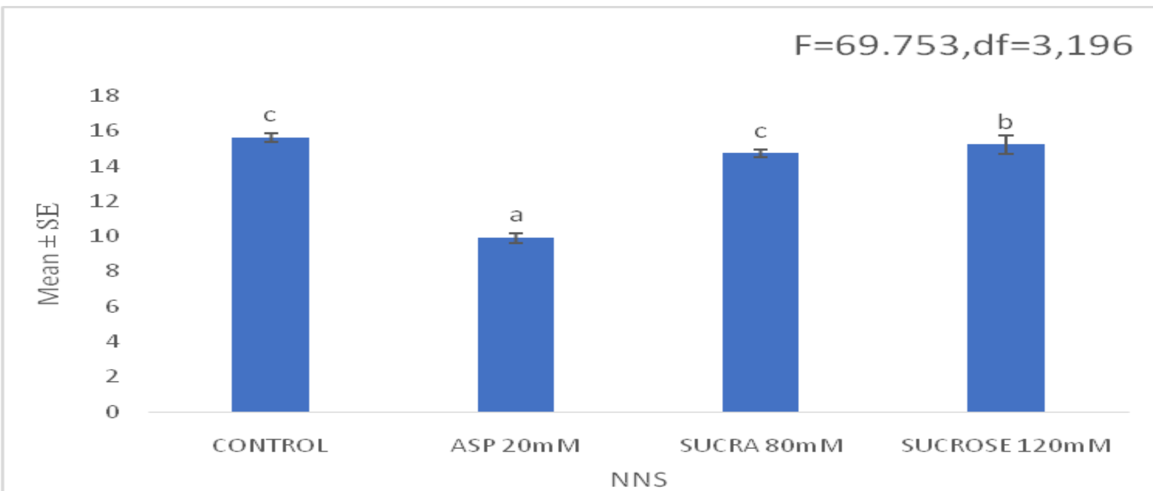


Different letters on the bar graph suggest significance byTukey's post-hoc test at the 0.05 level

Fig. 6: Mating latency of *D. melanogaster* in control and LD_{50} treated media (Aspartame/Sucralose/ Sucrose)

3.6. Mating activities and courtship patterns of *Drosophila melanogaster* flies when treated on different types of sweeteners media

Analysis of courtship activities of control and treated (Non-Nutritive and Nutritive Sweetener) flies showed a significant effect of added sweetener on the frequency of courtship behaviours (Table 1). Male courtship behaviours such as tapping, scissoring, vibration, licking and circling was highest in control flies and the least courtship activities were noticed in aspartame treated flies. Further among the treated flies, sucrose fed flies had greater courtship activities compared to female mating with sucralose and aspartame treated males. The least male courtship activities were noticed in aspartame treated flies. On the other hand, rejection responses by females such as extruding, ignoring, and decamping were highest in aspartame treated flies and least female rejection was found in control flies. Further among the treated flies female rejection was significantly lower in flies fed with sucrose compared to sucralose and aspartame treated flies. The results of a One-Way ANOVA followed by a Tukey's post hoc test on the data from male and female courtship activities revealed substantial differences between control and treated flies. Tukey's post hoc test revealed that male and female courtship behaviours differed significantly between control and treated media flies, as well as between treated media flies.



Different letters on the bar graph suggest significance by Tukey's post-hoc test at the 0.05 level

Fig. 7: Copulation duration of *D. melanogaster* in control and LD_{50} treated media (Aspartame/Sucralose/ Sucrose)

Table 1: Mating activities and courtship patterns of *Drosophila melanogaster* flies when treated on different types of sweeteners media

	Parameters	PAIRS: Aspartame (20mM) Male × Female	Sucralose (80mM) Male × Female	Sucrose (120mM) Male × Female	Normal Male × Female	F-Value
♂ courtship behaviors	No. of pairs	50	50	50	50	
	Tapping	10.9±0.162	12.4± 0.183	14.4 ±0.131	14.8±0.178	121.443***
	Scissoring	13.5±0.172	16.7±0.144	14.3±0.181	14.7±0.144	71.507**
	Vibration	10.5±0.115	11.3±0.129	11.6±0.131	14.5±0.160	168.348***
	Licking	7.2±0.167	9±0.128	9.8±0.140	13.5±0.146	330.074***
	Circling	11.6±0.131	14.6±0.146	17.4±0.114	17.6±0.131	464.333***
♀ Rejctn	Ignoring	15.4±0.171	14.2±0.318	13.6±0.265	10.3±0.157	84.475***
	Extruding	16±0.181	15.9±0.282	15.8±0.167	12.9±0.296	39.704**
	Decamping	19.4±0.194	18.7±0.256	18.5±0.308	17.9±0.243	5.955**

*Mean values are reported with SE; df=1,592, **P<0.01; ***P<0.001*

4. DISCUSSION

Studies on the influence of nutrient components on health, disease and survival have been observed using animal models by varying primary nutrient components such as sweetener, protein and fat [18]**.** These studies have shown that high sucrose diet has deleterious effects on health, survival and reproduction. Further, using rodent models have shown that a comparatively low level of added sweetener consumption has substantial negative effects on mouse survival, competitive ability, and reproduction [8]. Nowadays the use of nutritive sweetener can be restored with NNS [2]. To sensitively assess whether added NNS on *Drosophila* has negative health effects on toxicity, survival, climbing ability, mating behavior and mating success. Fig.1 supports the visual confirmation of food intake by the media in *D. melanogaster*. Behavioral studies, nutritional and drug administrations are essentially regulated by measurement of food intake under reliable conditions [19]. The mortality rate of *D. melanogaster* among different sweeteners shows that the mortality rate was significantly greater in NNS compared to nutritive sweetener sucrose. Additionally, among NNS, aspartame had significantly higher mortality than flies fed with sucralose (Fig. 2). Moreover, the mortality rate increased with increasing the concentration of each of the NNS studied revealed that studies in *D. melanogaster* with added NNS in the media has a significantly greater mortality rate, in other words, greater toxicity (Fig 3). This confirms earlier toxic studies of NNS [20]. The toxicity of sweeteners considered in the study is in the order of Aspartame> Sucralose>Sucrose. Both genetic and environmental factors are known to affect the toxicity of flies [21]. Toxicity varies between species and also different geographical populations of the same species of *Drosophila* [22]. Environmental factors such as flies age, cultural condition, photoperiod and diet used to culture the experimental flies are known to affect toxicity [23]. In the present study, the experimental stock consisting of the same age were used to be cultured in the same rearing conditions. Therefore, the observed toxicity was not due to physical factors, but because of the differences in the sweeteners used in the diet of the fly.

Flies general activity was also evaluated during the non-mating period using the climbing ability to understand the effect of NNS on their locomotion. Climbing ability also provides the details concerning their activity levels [24]. Activity levels such as food location, mating, predator escapes, and stress response are all important aspects of animal behaviour [25]. The present study also reveals that climbing ability was high in flies fed with sucrose compared to flies fed with NNS (Fig. 4). This suggests that energy is required for general avidity and also activity levels during courtship behaviour.

The reproductive success of *Drosophila* also depends on female receptivity and male activity during courtship [26]. The mating success of flies varies between species, between different strains [27]. In addition to this size, age and diet of the experimental flies also affect the mating success [28]. In the current research, as the experimental stock, their age and culturing parameters were all same and the only difference was the analysis of different sweeteners. Therefore, the observed variation in mating success is due to the effects of NNS. Fig. 5 illustrates that control flies had the highest percentage of mating success, followed by flies treated with sucrose-diet, and lowest percentage in aspartame treated flies. NNS flies mating success was much lower than that of nutritive sweetener flies in general. This confirms earlier studies of diet effects on mating success in *Drosophila* [28].

The success of mating also depends on the mating latency and activity levels of courting flies during mating [17, 29]. *Drosophila* courtship behaviour is broadly used to investigate coordination between male and female, sexual activity level and involves sensory processing (olfactory, visual and acoustic) [30]. Mating latency is the duration between male sexual activities till the initiation of copulation. Mating latency figures out the sexual activities of males and sexual receptivity of females in *Drosophila*. The courtship acts executed by males increase the females' receptivity to copulation [17, 31]. As the time is reverse of speed, in the present study, control flies had taken the least time to initiate copulation compared to flies treated with sweetener treated media. This suggests that NNS and nutritive sweetener may have more metabolic pathways to convert into energy for fly activity. Further among treated media, flies treated with Sucrose media took less time to initiate copulation when compared with flies treated with sucralose or aspartame media (Fig. 6). This indicates that flies that took less time to attract females are fast maters, whereas flies that took longer are slow maters. In the current research, Aspartame treated flies were slow maters whereas flies treated with control food were fast maters. This may suggest that aspartame treated flies have lower caloric values that led to starvation, inability of insects to digest and store energy of certain compounds, or its unappetizing nature than other sweeteners [32]. The present study evaluated the male and female courtship activities such as tapping, scissoring, vibration, licking, circling, ignoring, extruding and decamping. Male courtship behaviour was highest in control flies and the least courtship activities were noticed in aspartame treated flies. This is because *Drosophila* reproduction is influenced by chemicals released into the environment by food, predators, and mating pairs [25]. Further among the treated males, sucrose fed flies had greater courtship activities compared to sucralose or aspartame treated flies. The least male courtship activities were noticed in aspartame treated flies. On the other hand, rejection responses by females were highest in aspartame treated flies and least female rejection was found in control flies. Further among the treated flies, female rejection was significantly lower in flies fed with sucrose compared to sucralose and aspartame treated flies (Table 1). This suggests that male flies fed on NNS with less activity were not able to stimulate the female for mating whereas male flies fed with sucrose treated media/control food with their greater activity were able to convince and stimulate the counting female for mating. Therefore, their mating success was high. In species of *Drosophila,* mating success culminates in copulation [33]. Copulation duration is the time taken between initiation and termination of copulation. The copulation duration is also known to be influenced by genotype, environmental factors, size, age, etc. [34]. Copulation duration was shortest in aspartame treated flies and longest in control flies. Among the treated flies, sucrose treated flies copulated longer compared to flies treated with NNS aspartame and sucralose sweetener. Further, the least copulation duration was noticed in aspartame treated flies. The nutritional needs for courtship activities are often accompanied by the physiology and diet of flies. The chemical substances present in an individual fly affect the behaviour of other flies when released by their surrounding conditions. Chemicals produced by the source of food also act as signals for nutrient availability for sexual activities of the fly [25]. Thus, these studies suggest that control males with its fast-mating ability, greater courtship activities had convinced and stimulated females for mating and copulated longer than males treated with sweeteners. Further, males treated with NNS had fewer courtship activities had taken greater time for initiation of copulation and copulated shorter than males treated with nutritive sweetener sucrose. Furthermore, among the NNS's males treated with aspartame with its least courtship activities could not convince and stimulate the female for copulation and its copulation duration was shorter. As a result of these findings in *D. melanogaster*, it suggests that increased NNS had a negative impact on fitness traits such as climbing ability, courtship activities, and mating success.

5. CONCLUSIONS

Non-Nutritive Sweeteners are utilized as an alternative to sugar globally in their diets. Several NNS are existing worldwide with little or no empirical evidence to validate their efficacy. Hence, this research article emphasizes the effect of Aspartame and Sucralose on toxicity, physical activity and sexual behaviour of *D. melanogaster.* Aspartame treated flies were found to be highly affected in their mortality rates followed by sucralose and sucrose. After determining LD_{50} concentrations, climbing capacity and sexual activities were also found to be least in Aspartame treated flies when compared with NNS sucralose, nutritive sweetener sucrose and control. Sucralose and sucrose were seen relatively less effective in the physical activity and mating behaviour when compared with control. This data suggest that NNS are harmful when consumed in high doses and effects the physical activities and sexual behaviour of flies. Further studies and research works are required to examine the effect of Non-Nutritive Sweeteners at the molecular level in long term. Dose-dependent investigations and clinical trials on other organisms could be recognized in the future.

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Conflict of Interest

Authors report no conflict of interests.

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