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Effects of dried stevia leaves and stevia extract on the emergence pattern and survival of *Drosophila melanogaster*

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ABSTRACT:

Stevia is a zero calorie sugar that is natural origin that takes out from leaves of plant types *Stevia rebaudiana.* It is easily grown in the local area of Central America and South America. Based on previous information, author has hypothesized rational behind using stevia as low calorie sweetener and harmful effects (side effects), using a non-target model organism, *Drosophila melanogaster* (wildtype strain, Oregon R^{+}). Drosophila flies were reared on standard corn meal medium with yeast supplement and fed stevia dried leave and also powdered stevia (extract of stevia). Stevia dried leave and powder was also given along sulphur free sugar (which is standard component of *Drosophila* medium). Interestingly, flies fed stevia powder showed a significantly low emergence and reduced longevity as compared with sugar mixed group or normal food. Author have tested their result three independent time and found almost identical results. Conclusively, researcher can suggest a significantly lower emergence and survival in Drosophila melanogaster following treatment of alone stevia dried leave or stevia powder and this condition may be reversed with an addition of sugar supplement in the diet.

Keywords: *Drosophila melanogaster* (Oregon R+), Stevia dried leaves, Stevia powder (extract), Emergence, survival assay.

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INTRODUCTION

Stevia rebaudiana Linn., a plant belongs to the family of Asteraceae that is recognized with daisy plant and ragweed plant. The other names are sweet chrysanthemum, honey-leaf plant, sweet-leaf stevia, sugar-leaf or candy leaf and honey yerba etc. [1]. Stevia plant gets bigger 2-4 feet in height slightly; forked stems and increase completely above arrangement and certain depart of equatorial areas. For several years, the leaves and extract of stevia plant utilize for taste components. The soil should be dry red soil; sandy loam soil and pH scale is 6.5-7.5. Other soil like saline soil should be kept away from stevia plant [2]. Group of natural sugar for example; Diterpene glucosides have been taken out from stevia plant. Wild stevia plant leaves contains eight glycosides that involve stevioside and all units of rebaudioside. The most abundant of these constituents are stevioside and rebaudioside-A. Stevioside is a non-carbohydrate glycoside element. Steviol glycosides, it is a energetic constituent present in this plant that mostly formed stevioside and rebaudioside which have 30 to 50 times sweetener than normal sugar [3]. Heat and pH are firmly fixed and it is not fermentable [4]. It consist zero calories like other sugars, which is produced by human beings because the human digestive system does not break down glycoside in stevia. Moreover, some sterols are present in stevia plant and some antioxidant composited such as triterpenes, flavonoid, and tannins are involved. Like kaempferol, Quercetin, chlorogemic acid, caffeic acid, isoquercitrin, iso-steviol, etc are some taste formed polyphenolic anti-oxidant phytochemicals involve in stevia [5].

Stevia's taste has obtuse beginning and long lasting as compare to other sweeteners. Many sugar powders are bitter or licorice type after palate at excessive absorption. Stevia plant contains excessive freshness that is 95% steviol glycosides.

Leaves of stevia are small processed type, which is about 30-40 times sweeter than normal sugar. It is also a little bitter in taste. It can be used in tea, coffee and milk and also used in other hot and cold drinks Rebaudioside-A (extract of stevia) is found to be 300 times sweeter than normal sweetener It is less bitter as compare to stevia dried leaves. One gram of stevia powder is equal to two teaspoons of sugar. In this extract, there is zero glucose, zero carbohydrates and zero calories. Some distinctive effects in

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stevia powder have long ledge life and high inversion sufferance.

Stevia plant is been cultivated in many countries like Japan, China, Thailand, Paraguay, and Brazil in a large scale. In recent years, it has been prospering cultured in many states of India: Rajasthan, Maharashtra, Kerala and Orissa. In India, the high orders of natural sweeteners attract the farmers towards stevia cultivation in large scale [6]. In the European Union nation, *Stevia rebaudiana* and its goods are not utilized because of their assumed mutagenic possessions. Stevia leaf and stevia powder are imperfect to employ officially like a nutritional supplement in the United State of America. Conversely, a processed stevia glycoside is authorized in numerous of these nations for the utilization of rebaudioside-A.

Drosophila melanogaster is the most studied and well-accepted animal model organism [7]. High fecundity and short generation time are shown by flies and have developed fat body (mammalian adipose tissue analogues), cardiac anatomy, malphigian tubules (mammalian kidney analogous), central nervous system[2] [8-9], [10], [11], [12]. Drosophila as a capable model follows the demographic studies on different metabolic disorders, which provide efforts of inducing gene knockouts and mutagenesis [13].

Chandegra et al. [14], studied that more sugar diet results on feeding deportment, starvation resistance and lifespan on *Drosophila melanogaster*. Feeding reaction of flies may have secondary outcomes because feed production of female flies is less on such diet where male feed production is more. But female flies provide the capacity to inhibit starvation time from high sugar diets than male flies. It shows that female flies response to extra sugar, which may be increased towards surviving food deficiency in early stage. Female life span is more vulnerable to the harmful effect of more sugar diet at same time. May and his colleague [15], studied that the developmental food to establish fecundity, life history attributes and particular life time in fruit fly. The use of three different developmental nutrition stages, which consists large quantity of yeast and sugar: poor, rich, control. The development on low or large larval food enlarged the developmental time and for poor food, it reduced adult weight but it raised adult virgin life. It controlled the reproductive capacity of the adult environment with yeast. To determine the adult fecundity, the manipulation moved with larval food. Burke and Waddell et al. [16], studied on nutrient quality of sugar in *Drosophila*

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melanogaster. For animals, taste is the initial level in food and drink option. Observing sweetness represents the existence of sugar and viable caloric satisfaction. Some sugars cannot be necessary for life because sugary flavor can be undependable assumption of nourishing nutritious value. In mammals and in insect gustatory system, separate sugars are also observed by same sensible neurons that are different for animals to recognize distinct sugar taste singly. In case of Drosophila, recent study looks over the article of appetizing and relative nutritious value of sugars to memory development. Robust development formed by non-nutritious sweetness, which could be increased by tasteless appendages but nutritious substances. Within few minutes, a nutrient fact is passed to the brain when it can be used to conduct the appearance of sugar inclination memory. Using post-ingestive award assessment system, flies swiftly learn to distinguish between sugars and learn nutritious sugars. Demir et al. [9], studied on Drosophila to evaluate the genotoxicity of many sweeteners like aspartame, saccharin, acesulfame, sucralose in wing spot and comet assays. During process, it was used in two assays, which show sweeteners were not genotoxic. When trans heterozygous flies behave towards aspartame, they become low but it was important to become enlarge. In case of heterozygous flies, negative outcome were acquired because result created by aspartame are important due to somatic recombination in origin of mutant clones. In comet assay, aspartame shows genotoxic effect. Researcher made an attempt to evaluate the effects of stevia dried leaf and stevia extract on the development and survival of *Drosophila melanogaster*, an alternative to animal model.

MATERIALS AND METHODOLOGY

Experimental Animal:

Drosophila melanogaster, which is one of the most widely used and one of the most, understood of all the model organisms. Drosophila has a short, simple reproduction cycle about 8-14 days, which is influenced by room temperature. Researchers kept thousand of flies in the laboratory at the time and cheap to keep in laboratory. These flies require normal diet that is full of carbohydrates (corn meal) and proteins (yeast extract). wildtype *Drosophila* (Oregon R^{+}) was kindly gifted by Dr. Anurag Sharma, NITTE University, and Mangalore.

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Chemicals:

1) Stevia dried leaves
2) Stevia leaf extract

Stevia leaves and powder of stevia extract was procured from local market, Jalandhar.

Preservatives:

- Sodium benzoate parabene salt (0.5gm)
- Propionic Acid (0.5ml)

Methods for emergence pattern:

To evaluate the emergence of Drosophila flies, we took 48hrs (second instar larvae) under stereomicroscope. We took 10 larvae in each vial and 5 vials per group. As the normal food contains extracting solvent with different concentration of treatment chemicals such as stevia dried leaves along with sulphur free sugar (3.75gm) is 0.187gm, stevia powder along with sulphur free sugar (3.75gm) is 0.187 gm. In another groups, stevia dried leaves is 0.375gm and stevia powder is 3.75gm without using sulphur free sugar.

After 3-4 days of incubation, observe the groups of larvae and then count them without disturbing food. As larvae changes into pupae, count them carefully and allow them to grow properly. The number of flies emerging from different groups was recorded until all the flies emerged and record the date of emergence after 9-10 days [17].

Method for longevity assay using Drosophila:

After emergence of flies, the effect of stevia dried leaves and stevia powder was studies by treating the adult fly to food mix with these natural sweeteners along with different concentrations from Day 1 of their emergence.

Flies were transferred in vials, which contain control food and treatment food. Number of survival flies was recorded daily till the last fly was dead. This assay was continued until all the flies were dead [17].

Control food and treatment food is distorted every 2 days provided vials inserted into a cotton plug and count the number of dead flies.

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Lifespan measurement:

Analyze data to calculate mean lifespan and standard error. Plot survival curves between groups for mean lifespan. For all assays, statically significance difference are represented as * < 0.05 [8]. Maximum longevity study is conducted on the longest-lived 10% of flies in each treatment. $p<0.05$ were considered statically significant.

RESULTS

Emergence pattern of *Drosophila melanogaster*: The following graphs represent the emergence pattern of *Drosophila melanogaster* to check the additive effect of stevia leaves and stevia powder along with or without sugar. This experiment was done for three times and the result of these graphs is shown in the following three sets:

Figure 1 illustrates the emergence pattern of *Drosophila melanogaster* on five treatment groups (Treatment 1: control food, Treatment 2: sugar+ stevia dried leaves. Treatment 3: sugar+ stevia powder, Treatment 4: stevia leaves, Treatment 5: stevia powder) from Day 9 to Day 12. Overall, in case of control food, flies emerged more than treatment foods whereas least number of flies emerged in treatment 5 (stevia powder).

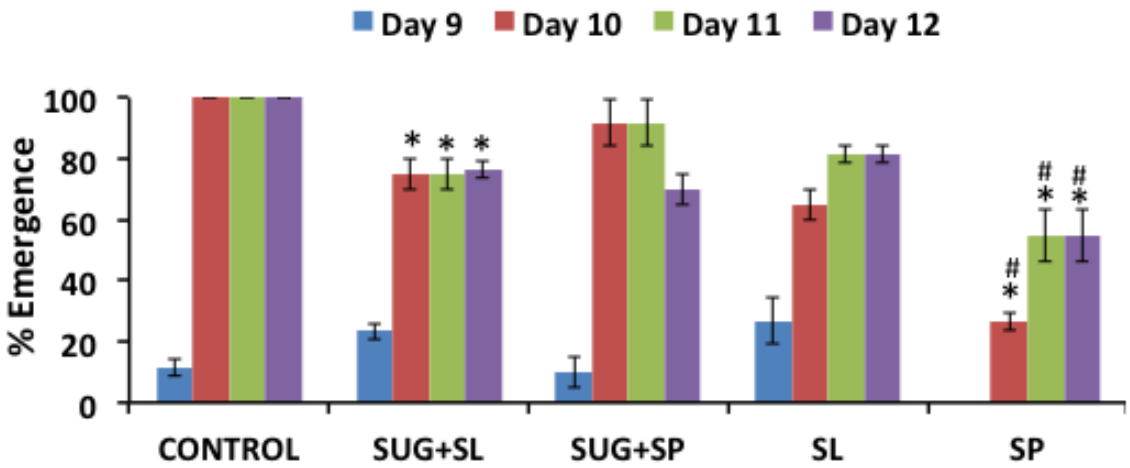

Figure 1: Plot of emergence pattern of wild type *Drosophila melanogaster* (Oregon R+) treated with Stevia dried leave, stevia powder and combinations. *Ascribed $P<0.05$ as compared with Control whereas # denotes $P<0.05$ as compared with SUG+SP.

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Survival of flies (longevity): As we see the above graph**,** it illustrates on five treatments (control food, stevia leaves along with or without sugar and stevia powder along with or without sugar) from day 1 (emergence of flies) to Day 39 (longevity of flies). Overall, in case of control food, flies emerged more on Day 1 and alive till Day 39 than other treatment groups whereas in stevia powder (without sugar) least no. of flies emerged on Day 1 and decreased the lifespan (alive till Day 27). Furthermore, in case of stevia powder (with sugar), flies emerged 100% on Day 1 and alive till Day 35 whereas in case of stevia leaves, flies emerged 80% on day 1 but alive till Day 36. On the other hand, flies emerged slightly less than 80% and alive till Day 36. To conclude, the longevity of flies was increased in control food but in case of stevia dried leaves and stevia extract along with or without sugar, stevia dried leaves along with or without sugar increased the longevity of flies (**Figure: 2**).

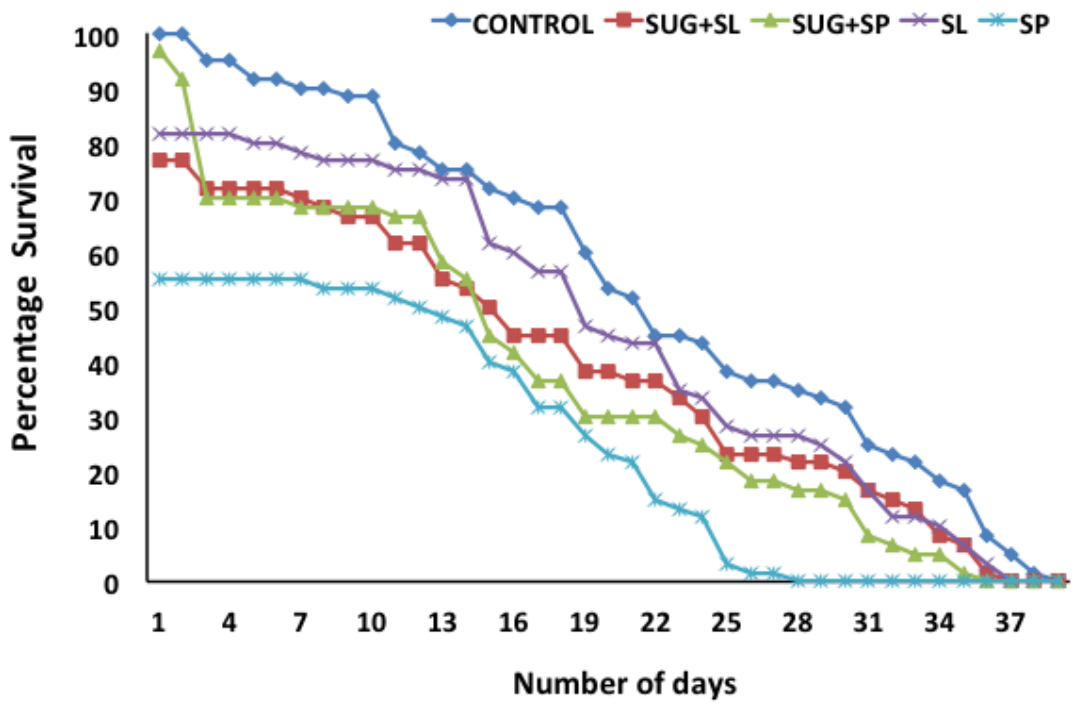

Figure 2: Plot of longevity of *Drosophila melanogaster* treated with stevia dried leave, powder and combinations with sugar. Significantly reduced survivals of flies were evident in Stevia powder

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and stevia leave treated groups as compared to control/normal food.

Discussion:

As we know, stevia is small persistent shrub that has been used as a bio-sweetener and utilized it for medicinal purposes for many times. Stevia plant leaves have useful and sensory properties whereas other high effectiveness sugar has not more advantages. It contains many steviol glycosides with high percentage of stevioside and rebaudioside A. The food preparation from rebaudioside-A permitted was passed from United States. The study of stevia plant was conducted on mosquitoes, mice and rabbit. In case of *Anopheles stephensi* (mosquito), in which stevia exhibited larvicidal potential and utilized for vector control to synthetic insecticide. This plant has potent antioxidative enzyme activity, which makes them strong from disease that occurred from free radicals [18]. In case of mice, stevia leaves, stevia extract and other goods decrease hepatic injury to the same amount and several variations produce on glucose and lipid metabolism [19]. As the replacement of normal sugar in yoghurt, individuals received the use of stevia powder in the ratio of 1:30 of extract to water from sensory testing [20]. In this report, researchers have investigated the role of stevia on *Drosophila melanogaster*.

As authors predicted based upon natural history of *Drosophila melanogaster* which is one of the most widely used and well studied of all the model organisms because it is easy to maintain and breed in the laboratory and has meticulous investigational benefits. In case of Drosophila flies, the effect of semolina-jaggery diet on the development and longevity was that it did not induce the weight of flies and lipid percentage .So this diet utilize for more development of healthy flies as compare to normal Drosophila diet [21].

In this report, we compared stevia as a natural sweetener with *Drosophila melanogaster*. The treatment chemicals like stevia dried leaves and stevia powder was given to the flies along with or without sulphur free sugar. We checked the role of

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dried stevia whole leaves on the development of *Drosophila melanogaster* and studied the role of extract of stevia on the development of flies. During procedure, we noticed the effect of stevia leaves or stevia powder along with or without sugar on the emergence pattern of Drosophila and judged the role of stevia in the longevity of *Drosophila melanogaster*.

In present study we find that on treating flies with stevia powder and stevia leaves, stevia leaves are more effective on the development and the longevity of the flies, but in comparison of control food with the stevia powder and stevia leaves, effect of control food is maximum, whereas emergence of flies were similar in the control food treated flies and stevia leaves treated flies. The effect of stevia powder with or without sugar did not showed any positive result, so we can say that stevia leaves remained more effective than the stevia powder.

Conclusion:

In this brief study, author concludes that the role of *Stevia rebaudiana* on the growth and longevity of *Drosophila melanogaster*. Author examined the role of stevia-dried leaves on the overall development of *Drosophila* and studied the role of stevia extract on the development of flies. Authors further investigated the additive effect of stevia-dried leaves and stevia powder along with or without sugar on the emergence pattern of flies and also judged the longevity pattern of flies. The longevity and emergence of flies was more in control food as compared to the treatment groups and reduced in stevia powder. We compared stevia dried leaves and stevia powder (with or without sugar), flies emergence and their longevity were increased in stevia dried leaves treatment groups. Therefore, we can say stevia leaves remained more effective than stevia powder.

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