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Thaumatin is similar to water in blood glucose response in Wistar rats

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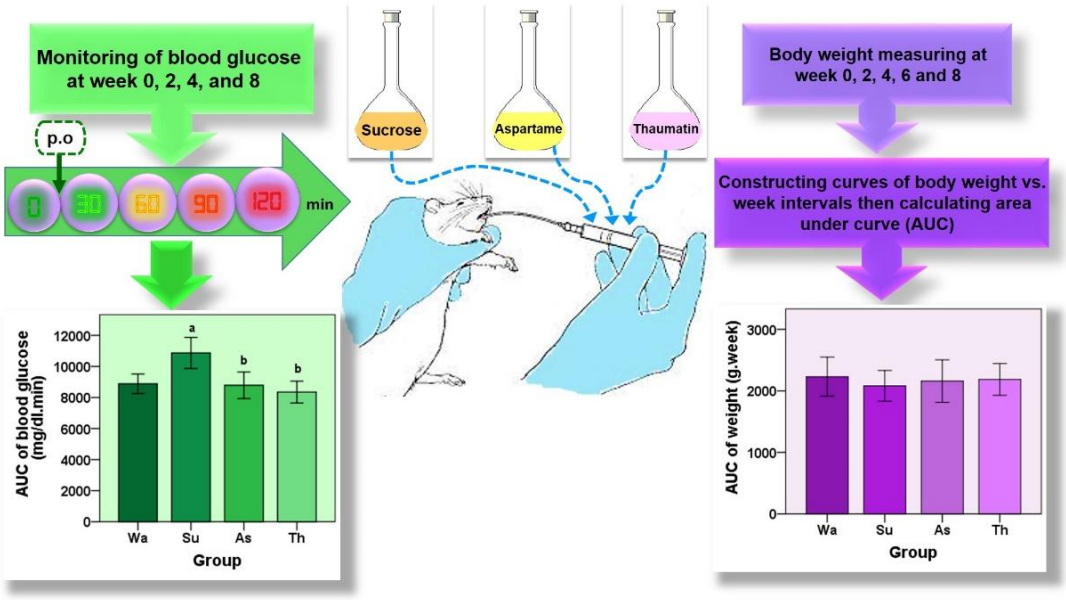
ABSTRACT:

The aim of this study was to investigate the effect of thaumatin; a sweet tasting protein, compared to a natural sweetener; sucrose and a synthetic one; aspartame on blood glucose level and body weight in male Wistar rats. Sucrose, aspartame, and thaumatin solutions have been administrated orally every day to adult male Wistar rats for 8 consecutive weeks. Within this period, blood glucose was monitored during two hours and body weight was measured every two weeks. It was found that compared to water, aspartame and thaumatin did not elevate blood glucose at all. Also, neither aspartame nor thaumatin affected body weight. Subsequently, thaumatin can be considered as a safer and healthier sweetener than aspartame or sucrose, because it is natural and do not cause elevating in blood glucose.

Keywords: *Blood glucose, Body weight, Sweetener, Thaumatin, Wistar rat*

GRAPHICAL ABSTRACT



I. INTRODUCTION

Sugar obtained from sugar cane or beet is widely used in many food products to give sweetness, viscosity, and consistency. Furthermore, it has a role in the preservation of foods [1]. However, its consumption has markedly increased in Europe and North America over the 19th and 20th centuries [2]. Unfortunately, the prevalence of obesity and type 2 diabetes is increasing globally. Obesity results from an imbalance between food energy intake and energy expenditure, which causing deposition of fat in subcutaneous and visceral adipose tissue [2,3],while diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in production of insulin by the pancreas or by ineffectiveness of the insulin produced [4].

Over the years a variety of potential causes for obesity have been posited, including increased carbohydrate consumption. Some studies in animals and humans have linked consumption of added sugars with prevalence of weight gain, obesity, and type 2 diabetes [5-7]. Therefore, there is a great need for sugar substitutes, which can help reduce caloric intake, in order to either reducing bodyweight in individuals having obesity or maintaining blood glucose levels in those with diabetes [8]. Artificial sweeteners like saccharin, aspartame, and acesulfame K are widely used as low calorie sweeteners because they are sweeter than sucrose several hundred times on a weight basis. Their contribution to the energy value of the food in which they are incorporated is negligible, since they produce the required sweetness in relatively low quantities [9]. But on the other side, they have side effects such as psychological problems, mental disorders, bladder cancer, heart failure, and brain tumors [1,4]. Recently, there have been studies that linked the use of artificial sweeteners with the increased risk for obesity and type 2 diabetes in genetically susceptible persons through impacting the host physiology and decreasing beneficial bacterial species [10].

Non-saccharide natural sweetening agents are preferred over synthetic ones since they do not have any adverse impact on health, are low caloric, nontoxic and super sweet in nature and hence can overcome the problems of sucrose and synthetic sweeteners [1,11]. Thaumatin is one of the sweet tasting proteins permitted for food use in the European Union (EU). It is obtained from the fruit of the plant *Thaumatococcus daniellii* (Benth.), metabolized as other proteins, and is 2000-3000 times sweeter than sugar on a weight basis without an ADI limit [9]. There have been many studies on this sweetener including structure-sweetness relationship [12,13], and stability [14], but effect on blood glucose was not studied in detail like this experiment. Although many intense sweeteners have been tested for their effects on blood glucose in healthy and diabetic individuals in a large number of studies, thaumatin was not incorporated in anyone of them [8,15-17].

The present study was designed to evaluate the effect of the sweet tasting protein thaumatin versus some common sweeteners; sucrose as a natural sweetener and aspartame as a synthetic one, on blood glucose level and body weight in normal Wistar rats. Doses have been calculated to be equivalent in sweetness to the average of total amount of sugar consumed daily according to a previous statistics [18]. Thus, these doses will give us an idea about the studied effects if thaumatin would substitute all sugar quantity consumed daily.

II. MATERIALS AND METHODS

2.1. Materials

Thaumatin (**Th**) was purchased from Naturex (England). Aspartame (**As**) and Sucrose (**Su**) were obtained from Fooding Group Ltd. (China) and Scharlau Chemie S.A. (Spain), respectively. Sweeteners were freshly prepared in double distilled water (**Wa**) that was produced in our laboratory by Janat Instruments (Syria).

2.2. Animals

Adult male Wistar rats, weighing 220 - 300g, were used in this study. The experimental animals were

purchased from the animal house of Research Center, Damascus, Syria. Animals were housed in standard plastic cages and maintained under controlled laboratory conditions of temperature (20 ± 2°C) and a 12:12 h light/dark cycle. Rats were fed ad libitum on normal commercial chow and had free access to water. Experiments were carried out in accordance with the Ethical Guidelines for the Use of Animals in Research; and always started at the same hour (9 am.). Efforts were made to minimize animal suffering.

2.3. Experimental design

A total of 40 rats were randomly divided into 4 groups of 10 rats each (n=10). Group 1 was treated with only water (**Wa**) at an average of 0.65 ml/day and served as control. Group 2 was treated with sucrose (**Su**) as a reference sweetener at a dose of 687.143 mg/kg, while the remaining two groups were treated with aspartame (**As**) and thaumatin (**Th**) at 3.436 mg/kg and 0.275 mg/kg, respectively. Water and all mentioned sweeteners were given by oral gavage (p.o.) as single daily treatments for 8 consecutive weeks. These doses were selected based on a previous statistics estimated daily sucrose intake [18], then doses were adjusted to rat's body weight while giving equivalent sweetened solutions.

2.3.1. Monitoring blood glucose level (BGL)

After an overnight fasting, blood samples were withdrawn from tail vein by puncture [19] and BGL was measured using an Accu-Chek ® Active glucometer (Roche, Mannheim, Germany), indicating zero time of the test. Water (**Wa**) or sweeteners were given orally each at its corresponding dose. Then, BGL was also determined at 30, 60, 90 and 120 min after treatment receiving. This whole procedure was carried out at baseline, 2, and 4 weeks after. Curves of BGL (mg/dl) versus time intervals (min) were constructed and area under the curve (AUC) was calculated by the trapezoidal method.

2.3.2. Studying body weight

For all rat groups, the body weight was measured at baseline and every two weeks after. These weights were determined at the same time during the morning before starting the experiments, by using a digital balance (SI-132, Excell). Also, AUCs were calculated as mentioned before.

2.4. Statistical analysis

Data were expressed as mean ± standard deviation (SD). Analysis of data were performed with IBM® SPSS® statistics software v.20 using repeated measures analysis of variance or one-way analysis of variance (ANOVA) as appropriate, each followed by Tukey's test for multiple comparisons. These tests were used to determine whether there were significant differences either in blood glucose concentrations or body weight between sweeteners and water or sucrose, and to study changes over time compared to baseline value. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of sweeteners on blood glucose level (BGL) in male rats

For **Su** group, a significant elevating in BGL occurred as expected at 30 min (peak time) after oral gavage as shown in Table 1. Then, BGL start decreasing to the end of test, but remained significantly higher than control. Whereas **As** and **Th** groups failed to change it significantly and were just like control group ($p > 0.05$). Furthermore, during the two hour test period, BGL did not change significantly from baseline value in all groups except for **Su** that had significant changes from 30 to 120 minute measurements. These observations had repeated at all week periods; baseline, 2, and 4 weeks after. Subsequently, BGL did not return to its fasting level in **Su** group unlike others whose BGL values remained unchanged throughout the whole test time.

Table 1. Blood glucose at week 0 during two hours after oral gavage

Group	Blood glucose (mg/dl)				
	0 min	**30 min**	**60 min**	**90 min**	**120 min**
Wa	78.1 ± 11.6	81.6 ± 5.2	80.3 ± 4.6	78.6 ± 10.7	79.4 ±6.0
Su	80.6 ± 10.1	109.1 ± 16.6$^{***,\#\#\#}$	101.9 ± 13.7$^{***,\#\#}$	103.3 ± 7.7$^{***,\#\#\#}$	97.7 ±16.1$^{**,\#\#\#}$
As	75.9 ± 15.0	81.6 ± 8.0†††	79.9 ± 8.8†††	79.5 ± 5.9†††	79.3 ±10.6††
Th	71.7 ± 8.4	71.7 ± 8.4†††	68.1 ± 5.2†††	72.1 ± 7.8†††	72.3 ±8.0†††

Data are expressed as mean ± SD, n = 10. **Wa**: Control, **Su**: Sucrose, **As**: Aspartame, **Th**: Thaumatin.
** $p < 0.01$ vs. control. *** $p < 0.001$ vs. control. †† $p < 0.01$ vs. sucrose. ††† $p < 0.001$ vs. sucrose. $^{\#\#}$ $p < 0.01$ vs. corresponding baseline value. $^{\#\#\#}$ $p < 0.001$ vs. corresponding baseline value.

Fig.1 represents AUC values for blood glucose curves as an indicator of the overall effect of sweeteners on BGL within the two hour test period. Again, **Su** group had the highest value among the groups whereas AUC values of **As** and **Th** groups were lower than **Su** value significantly. The presented results indicated that both **As** and **Th** have acted the same and did not elevated BGL since they are a dipeptide and a protein respectively. Regarding insulin secretion, it is known that amino acids can promote this phenomena [20]. However, in our experiment, we can assume that both amino acids resulted from aspartame or thaumatin digestion and then absorption did not promote insulin secretion because neither concentrations of blood glucose decreased significantly from control, nor over time compared to baseline values, as demonstrated in Table 1. This can be due to the minute amount used daily of **As** or **Th** (just about 1mg).

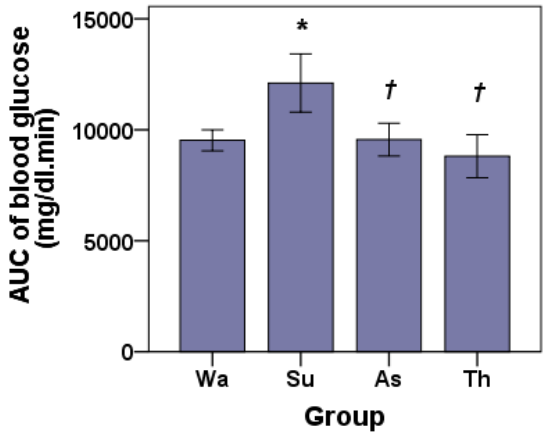


Fig.1 Area under the curve ($AUC_{0\text{-}120min}$) of blood glucose at week 0

Data are expressed as mean ± SD, n = 10.**Wa**: Control, **Su**: Sucrose, **As**: Aspartame, **Th**: Thaumatin.
* $p < 0.001$ vs. control.† $p < 0.001$ vs. sucrose.

3.2. Effect of sweeteners on body weight in male rats

As shown in Table 2, body weight of **Su** group was not significantly different from control group at any week period. Also, **As** and **Th** groups were similar to control in body weight at all week periods ($p > 0.05$). On the other hand, body weight of all sweetener groups have increased gradually from baseline until the end of the 8 week period just like control one. Although body weight in **Su** and **Th** groups was significantly higher (at least

$p < 0.01$) than their baseline values starting from 2nd week (weight gain percentage was 9.2 ± 3.4 and 8.5 ± 2.4 %, respectively), this was not observed in **Wa** and **As** groups almost until the end of experiment (weight gain percentage was 6.6 ± 5.0 and 4.4 ± 5.9 %, respectively).

Table 2. Body weight of male rats at all week intervals

Group	Body weight (g)*				
	Week 0	**Week 2**	**Week 4**	**Week 6**	**Week 8**
Wa	269.9 ± 35.4	275.2 ± 41.9	278.0 ± 41.4	283.7± 40.2 ††	287.6 ± 37.8†††
Su	248.8 ± 29.3	255.5 ± 32.3††	258.4 ± 31.8†††	266.7 ± 31.3†††	271.6 ± 32.0†††
As	263.9 ± 37.7	266.4 ± 45.2	270.3 ± 46.3	273.1 ± 44.9	275.4 ± 39.3†††
Th	261.3 ± 30.1	270.1 ± 33.2††	272.7 ± 32.7††	277.7 ± 32.2†††	283.4 ± 32.7†††

Data are expressed as mean ± SD, $n = 10$. **Wa**: Control, **Su**: Sucrose, **As**: Aspartame, **Th**: Thaumatin.
* Sweetener groups for each week period, are not significantly different from control group ($p > 0.05$).
$^{\dagger\dagger}p < 0.01$ vs. corresponding baseline value. $^{\dagger\dagger\dagger}p < 0.001$ vs. corresponding baseline value.

After drawing weight vs. week intervals curves, AUC has been calculated to evaluate the overall effect of sweeteners on body weight of rat during the whole experiment period. AUC value for either **Su**, **As**, or **Th** group has not been statistically different from control ($p > 0.05$) as shown in Fig.2.

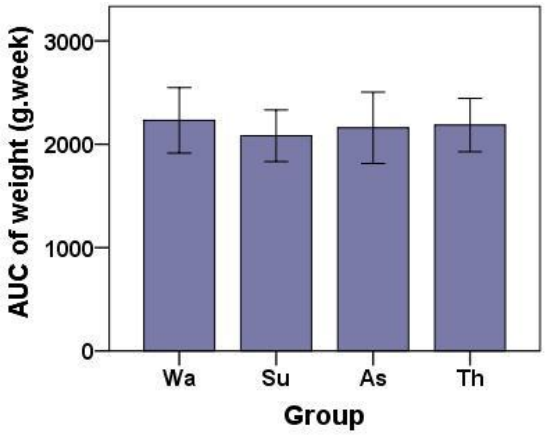


Fig.2 Area under the curve ($AUC_{0\text{-}8week}$) of body weight after 8 weeks*

Data are expressed as mean ± SD, $n = 10$. **Wa**: control, **Su**: sucrose, **As**: aspartame, **Th**: thaumatin.
* Sweetener groups are not significantly different from control group ($p > 0.05$).

Results clearly have shown that sweeteners themselves had no effect on total weight gain percentage ($p > 0.05$). However, there have been some insignificant differences that can be due to that sucrose is a known carbohydrate source and his content of glucose can be used later to form glycogen or even lipids. While thaumatin in spite of being used in an amount less than aspartame (12.5 times) is a high quality protein with 207 residues from 17 different amino acids, and body can use them to synthesize needed proteins or are converted to lipids. Finally, aspartame which is just a small dipeptide, provides body only with 50% phenylalanine (an essential amino acid) by weight of dose which explains the slow increasing in body weight of **As** group [9, 21-23]. Although doses used in this experiment were equivalent in sweetness to the average daily amount ingested

by humans of sucrose, the caloric content of dose used of **Su**, **As**, and **Th** was relatively very low (2.75, 0.014, 0.0011 cal/kg/day, respectively) and can be considered to have no significant contribution to the caloric daily intake, hence to body weight gain. Subsequently, we can conclude that these sweeteners themselves had no effect on body weight of male Wistar rats using the mentioned doses.

III. CONCLUSION

This study has demonstrated that ingestion of thaumatin for consecutive 8 weeks at the used dose level, had neither effect on blood glucose level during two hour test, nor on body weight of male Wistar rats. Aspartame in addition to have low sweetness intensity as compared to thaumatin, it has many side effects as mentioned before, synthesized chemically, and its intake should not exceed its ADI limit (40mg/kg/day) [9]. However, thaumatin does not have the previous disadvantages and so, it can serve as a safe natural super alternative sweetener. In future, there will be a great need to further studies in order to expand knowledge of these sweeteners, especially thaumatin and understanding their other effects on experimental animals or human volunteers.

Conflicts of interest

The authors declare no conflicts of interest.

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