

# Sacoglottisgabonensis Aqueous Extract reduces Weight Gain in Aspartame-Exposed Swiss Mice".

## ABSTRACT

**Aim:** To evaluate Sacoglottisgabonensis aqueous extract reduces weight gain in Aspartame-exposed Swiss Mice".

**Study Design:** The study was a completely randomized design employing relevant statistical tools for analysis and interpretation.

**Place and Duration of Study:** The study was carried out in the Department of Animal and Environmental Biology, Rivers State University. The experiment lasted for 90 days between April and June 2022.

**Methodology:** A total of 30 adult male Swiss mice (mean weight 23.07±0.12g) were divided into six groups of five animals each. Group A was the negative control which received neither Sacoglottisgabonensis nor Aspartame, group B was the positive control which received 50mg of Aspartame only, group C received 50mg of Aspartame with 250mg of aqueous bark extract of S. gabonensis, group D received 50mg of Aspartame with 250mg of aqueous leaf extract of S. gabonensis, while groups E and F received 50mg of Aspartame with a combination of 250mg and 500mg of extracts of S. gabonensis bark and leaf extracts respectively. The experimental animals were weighed twice weekly for 30, 60 and 90 days experimental duration and recorded to the nearest 0.01g.

**Result:** There was significant increase (p<0.05) in weight in the 30, 60 and 90-days exposure periods in group B. The groups that received the combination of aspartame and S. gabonensis particularly at higher doses showed a significant decrease in weight across the exposure periods when compared to the group that received aspartame only and the control group.

**Conclusion:** This finding suggests that S. gabonensis reduced aspartame-induced weight gain, indicating its potential benefits for weight management. Further research is needed to explore the underlying mechanisms and health implications.

**Keywords:** Extract, Sacoglottisgabonensis, sweeteners, weight gain

## Introduction

Majority of people all over the world consume foods containing one sweetener or the other [1]. Artificial sweeteners are used worldwide as sugar substitutes in dietary soda, chewing gum, ice cream, breakfast cereal and most sugar free foods. It is also used in food products including dry beverage mixes, chewable multi-vitamins, breakfast, puddings and fillings, carbonated beverages, refrigerated and non-refrigerated ready to drink beverages, yogurt type products and pharmaceuticals [2,3]. Aspartame is an artificial (non-nutritive) sweetener used to replace sugar in food and drinks [4]. It is an odourless, white crystalline powder with a clean, sweet taste without any aftertaste or cooling effect [5]. Aspartame is one of the most widely used artificial sweeteners in the world. It is found in more than 6000 products [6]. The accepted daily recommended dose by the FDA is 50mg/Kg body weight/day while the

**Comment [GA1]:** How many days were the treatments applied to each group?

**Comment [GA2]:** Rewrite please. The authors should clarify the weighing frequencies. Do the 30, 60 and 90 days weighings concern the same batches of Swiss mice?

**Comment [GA3]:** How was the 30, 60 and 90 day treatment carried out within group B?

**Comment [GA4]:** Which ones?

**Comment [GA5]:** Replace with 'this result'

**Comment [GA6]:** The objectives of this study are not clearly defined. The last sentence of the introduction is more about the problem of the study.

**Comment [GA7]:** Be more explicit. Do you suggest any natural or artificial sweeteners?

accepted or/and approved daily dose by the World Health Organization (WHO) is 40mg/kg/day [7]. Although there is concern suggesting possible adverse neurological and behavioural effects due to aspartame's metabolic components phenylalanine, aspartic acid (aspartate), diketopiperazine and methanol, which are produced during its breakdown [8]. Aspartame represents 62% value of the intense sweetener marketed and consumed worldwide in over 100 countries [9,10].

*Sacoglottisgabonensis* is a tree found in the tropical rainforest region of Africa and America is commonly used as an additive to palm wine, a local alcoholic brew which is an exudate from the phloem of *Raphia* species and *Elaeisguinensis* [11,12]. *S. gabonensis* extract is used as a stimulant, pain killer, the sap is medicinal and prolongs the shelf-life of fresh palm wine. The stem bark extract is taken to treat fever, diarrhoea, gonorrhoea, abdominal pain, and as a spice in food to induce heat in nursing and pregnant mothers in Sierra Leone [13,14]. The stem bark and the leaf extract reduced altered electrolytes concentrations in Swiss mice [15,16]. It is reported to have aphrodisiac properties. [17]. Several reports have linked aspartame to weight gain, obesity, increased appetite. [18] reported increased body weight and fat mass mainly due to an increase in energy efficiency in rats exposed aspartame and sucralose while [19] also suggested a long-term intakes of aspartame, saccharin or diet soda may increase adipose tissue deposition and risk of incident obesity independent of diet quality or caloric intake. [20] reported that BMI is positively associated with the consumption of diet carbonated beverages and people who regularly consume artificial sweeteners are at increased risk of excessive weight gain, metabolic syndrome, type 2 diabetes and cardiovascular disease [21]. Therefore, there is need to evaluate and source for medicinal plant to help reduce the overall weight gain of individuals with incessant consumption of aspartame.

## 2. Materials and Methods

A total of 30 adult male swiss mice (mean weight 23.07±0.12g) were used for the study. All experimental animals were allowed to acclimatize for two weeks during which animals were allowed access to clean water and rodent pellet *ad libitum* before the commencement of treatments. The experiment lasted for 90days. Dried leaves and bark of *S. gabonensis* were homogenised using kitchen blender. A tincture of both extracts was administered to the experimental animals at the dosage of 250 and 500 mg/kg/bw/day. 50mg of aspartame was used according to the recommendation of the FDA.

The animals were divided into six groups of five each. Group A was the negative control and so received water and feed only. Group B was the positive control which received 50mg /kg/bw/day of aspartame only, group C received 50mg/kg/bw/day of aspartame with 250mg/kg/bw/day of bark extract of *S. gabonensis*, group D received 50mg/kg/bw/day of aspartame with 250mg of leaf extract of *S. gabonensis*, while groups E and F received 50mg/kg/bw/day of aspartame each with 250mg/kg/bw/day and 500mg/kg/bw/day of combined extracts of *S. gabonensis* respectively. All treatments were administered by oral gavage and followed the institutional animal care and treatment protocols laid down as approved guidelines for the ethical treatment of laboratory animals and at the Rivers State University. The weight of the experimental animals were taken twice weekly for 30, 60 and 90 days using a digital balance (Model PCE-BTS 15) and recorded to the nearest 0.01g. The experimental data were subjected to descriptive statistics that include means (M), standard deviation (SD), and standard error (SE) of means. Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4.

## 3. Results and Discussion

**Comment [GA8]:** There is no connection between *Sacoglottisgabonensis* and the preceding paragraph. The authors must raise a problem which links *Sacoglottisgabonensis* to the previous paragraph. They could mention the search for an inhibitor of the effects of artificial sweeteners in the body.

**Comment [GA9]:** This part should be included in the previous paragraph

**Comment [GA10]:** Why the fixation on medicinal plants? Couldn't other types of plants be useful? I suggest that the authors focus on finding a solution from natural plants.

**Comment [GA11]:** The authors should have used statistical tests for more rigorous results. Repeated measures ANOVA, Analysis of covariance (ANCOVA) or other equivalent tests could have been used.

**Comment [GA12]:** Why ignore the WHO recommended doses that you mentioned? How does this choice explain? The FDA recommends 50 mg/kg body weight/day, while your mice have an average weight of 23.07±0.12g. I think this is clearly an overdose by FDA standards. How do the authors explain this methodological approach?

**Comment [GA13]:** The authors should really clarify the notion of 30, 60 and 90 days. If there are 2 weight measurements per week, how many weight measurements were taken on each group throughout the study?

**Comment [GA14]:** The authors should have used statistical tests for more rigorous results. Repeated measures ANOVA, Analysis of covariance (ANCOVA) or other equivalent tests could have been used.

**Comment [GA15]:** The results suggest the use of statistical tests, including comparison tests that are not discussed in methodology.

### 3.1 Effects of aspartame and *Sacoglottisgabonensis* on Body weight of Swiss Mice.

The effects of aspartame and *S. gabonensis* on the mean body weights of each group of the experimental animals exposed for 30 days is shown in table 1. There was a significant difference ( $p < 0.05$ ) in weekly weights of mice within treatment groups. A significant ( $p < 0.05$ ) increase was observed within the first 30 days of the experiment in group B that received aspartame only. However, there was no significant ( $p > 0.05$ ) difference in weekly weight of mice from the group D (that received aspartame and bark extract of *S. gabonensis*), group E (that received aspartame 50mg/kg/bw/day and 250mg/kg/bw/day of combined extract of *S. gabonensis*) when compared to the control group at ( $p < 0.01$ ) and group F (that received aspartame and 500mg/kg/bw/day of combined extract (bark and leaf) of *S. gabonensis*)

**Comment [GA16]:** Is this a comparison test? This must be specified in the methodology.

**Comment [GA17]:** Is this a comparison test? This must be specified in the methodology.

**Table 1: Comparisons of Weekly Weights of Swiss Mice after 30 Days administration of Aspartame and *Sacoglottis gabonensis***

	Body Weight (g)			
	Week 1	Week 2	Week 3	Week 4
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
<b>Group A</b>	24.99±0.34	26.68±0.12	29.35±0.75	29.09±0.83 <sup>b</sup>
<b>Group B</b>	24.04±1.25	27.93±2.38	30.76±2.07	32.27±1.97 <sup>a</sup>
<b>Group C</b>	23.73±0.83	25.76±0.78	29.13±1.71	29.38±1.32 <sup>b</sup>
<b>Group D</b>	23.62±2.57	25.36±3.41	26.87±2.80	29.25±4.04 <sup>b</sup>
<b>Group E</b>	24.12±1.41	26.84±1.39	28.58±1.22	29.78±1.13 <sup>b</sup>
<b>Group F</b>	23.39±0.94	25.76±1.10	26.28±1.69	28.43±1.70 <sup>ab</sup>
<b>F- ratio</b>	2.15	0.54	2.43	6.79
<b>P-value</b>	0.1720 <sup>ns</sup>	0.6695 <sup>ns</sup>	0.1405 <sup>ns</sup>	0.0137 <sup>s</sup>

\*Values with the same superscripts are not significantly different @ $p > 0.05$ . However, Values with different superscripts are significantly different @  $p < 0.05$ .

The comparisons of weekly weights of Swiss mice after treatment with aspartame and *Sacoglottisgabonensis* (week 5-8) is presented in Table 2. There was increase in weight gain but no statistical significant difference in weekly mean weight values of all the experimental animals.

**Table 2: Comparisons of weekly weights of Swiss mice after treatment with Aspartame and *Sacoglottisgabonensis* (Weeks 5-8)**

Treatment	Weight (g)			
	Week 5	Week 6	Week 7	Week 8
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
<b>Group A</b>	29.47±2.25 <sup>ab</sup>	30.76±2.47 <sup>c</sup>	30.46±1.50 <sup>c</sup>	30.76±0.95 <sup>c</sup>
<b>Group B</b>	34.07±0.85 <sup>a</sup>	34.96±0.08 <sup>a</sup>	35.65±0.44 <sup>a</sup>	39.93±0.18 <sup>a</sup>
<b>Group C</b>	29.24±0.60 <sup>ab</sup>	29.77±0.96 <sup>bc</sup>	29.76±1.40 <sup>bc</sup>	29.66±1.88 <sup>c</sup>
<b>Group D</b>	30.52±0.89 <sup>b</sup>	29.78±0.62 <sup>bc</sup>	29.86±1.28 <sup>bc</sup>	30.34±1.47 <sup>c</sup>
<b>Group E</b>	31.80±3.99 <sup>b</sup>	31.51±3.99 <sup>c</sup>	31.91±3.59 <sup>b</sup>	31.14±3.69 <sup>b</sup>
<b>Group F</b>	31.97±1.18 <sup>b</sup>	32.39±0.75 <sup>b</sup>	32.23±0.92 <sup>b</sup>	32.17±2.27 <sup>b</sup>
<b>F-Ratio</b>	<b>2.67</b>	<b>3.59</b>	<b>2.70</b>	<b>2.91</b>
<b>P-value</b>	<b>0.0431<sup>s</sup></b>	<b>0.059<sup>s</sup></b>	<b>0.02619<sup>s</sup></b>	<b>0.0302<sup>s</sup></b>

\*SE: Standard error, s=Not Significant (p>0.05).

The comparisons of weekly weight of Swiss mice after treatment with aspartame and *Sacoglottisgabonensis* between week 9 to 12 showed significant (p<0.05) difference in weight gain from week 10 to week 12, while there was non-significant(p>0.05) difference in weight gain in week 9 across treatment groups.

Table 3 Comparative analysis of Weekly Weights of Swiss Mice after Treatment with Aspartame and *Sacoglottisgabonensis* (Weeks 9-12).

Treatment	Weight (g)			
	Week 9 Mean ± SE	Week 10 Mean ± SE	Week 11 Mean ± SE	Week 12 Mean ± SE
Group A	31.26±0.20 <sup>ad</sup>	32.85±0.45 <sup>ad</sup>	32.12±0.40 <sup>a</sup>	33.28±0.69 <sup>a</sup>
Group B	40.22±1.19 <sup>a</sup>	40.54±0.63 <sup>bc</sup>	41.09±1.13 <sup>c</sup>	41.91±0.47 <sup>ab</sup>
Group C	30.07±0.63 <sup>b</sup>	31.00±0.63 <sup>bc</sup>	31.47±0.65 <sup>a</sup>	32.59±0.91 <sup>bc</sup>
Group D	31.76±1.45 <sup>ad</sup>	30.05±0.46 <sup>c</sup>	29.82±0.72 <sup>a</sup>	30.78±0.91 <sup>c</sup>
Group E	32.32±0.89 <sup>ab</sup>	32.96±0.69 <sup>ab</sup>	32.50±0.42 <sup>ab</sup>	32.86±0.10 <sup>bc</sup>
Group F	32.70±0.55 <sup>ab</sup>	35.23±0.35 <sup>a</sup>	34.06±0.63 <sup>a</sup>	34.84±0.61 <sup>d</sup>
<b>Test Statistics: F-Ratio</b>	2.254	9.330	10.223	8.141
<b>P-value</b>	0.0215 <sup>s</sup>	0.0008 <sup>***</sup>	0.0005 <sup>***</sup>	0.0015 <sup>**</sup>

SE: Standard error, Significance Level: \*\*=p<0.01; \*\*\*=p<0.0001; ns=Not Significant (p>0.05). Superscript with the same alphabet showed non significant difference while Superscripts with different alphabet showed significant difference.

Changes in body weight is major criterion used for the evaluation of potential systemic toxicity of harmful chemicals and toxicants in living system [22]. There was no significant (p>0.05) difference in weight across treatment groups from the initial weight to the final weight within the 30days exposure period in the groups that received the both leaf extract and 50mg/kg/bw/day of aspartame and 250mg/kg/bw of *S. gabonensis*bark extract compared with the control group. The groups that received aspartame along with 500mg/kg/bw combined bark and leaf extract of *S. gabonensis* and group B which received aspartame only showed no significant difference in weekly weight gain

Administration of aspartame and *Sacoglottisgabonensis* to experimental mice from weeks 5-8 is shown in table 2. There is no significant difference (p>0.05) in weight in group C compared to group A. However, a significant increase occurred in group B administered aspartame only compared with other groups coadministered *S. gabonensis* and the control. The body weights of Swiss mice after treatment with aspartame and *Sacoglottisgabonensis* in groups D,E and F showed no significant increase compared to the control group. This trend was also seen after 90days administration,

Group B, the positive control group had the highest weight gain which was statistically significant while the other groups had lower weights despite coadministration with either leaf, bark or combination of both extracts. This result implies that aspartame influenced weight gain by inducing hyperphagia while the other treatment groups and negative control group still maintained steady decrease compared with group B. The extracts of *S. gabonensis* reduced weight gain when compared with group B administered aspartame only.

Similar observation was made by [23,24] who reported increased weight gain and total feed intake in rats administered monosodium glutamate alone. [24,25] also recognized various medicinal plants and herbal decoction of *Hibiscus rosa sinensis* flower and *Zingiber officinale* as a natural product source and therapeutically effective against overweight and obesity due to fewer side effects in comparison to synthetic drugs.

The impact of aspartame on body weight in Swiss mice suggests that, at least in short-term studies, aspartame does not consistently lead to weight gain but in a long term consumption. Moreover, this result indicates that the effects of aspartame might be influenced by multiple factors, including dosage and duration of exposure. Administration of the extracts of *S. gabonensis* to experimental mice has proved to be beneficial as it maintained a steady weight throughout the experimental period.

#### 4. Conclusion

Comparison of weight gain of Swiss mice exposed to aspartame and *Sacoglottisgabonensis* for 30-, 60- and 90-days exposure periods strongly suggest that aspartame influenced feed intake that led to the observed increase in weight in the group that received aspartame alone, while the groups that received the extracts of *S.gabonensis* showed slow but steady growth, which significantly decreased compared to group B. This implies that *S. gabonensis* moderated weight gain in experimental mice and can serve as an additive for those who are at risk of developing increased body weight due to environmental factors and genetic predispositions.

**Comment [GA18]:** Each time you write this expression differently. you have to harmonize.

#### Reference

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