Reprint from The Review of DIABETIC STUDIES Vol 20 No 1 2024

The Review of DIABETIC STUDIES

RESEARCH

Commercial Non-nutritive Sweeteners Vitiated Redox Imbalances in in Vivo Rat Model

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Manuscript Submitted March 01, 2024; Resubmitted April 09, 2024; Accepted April 17, 2024

■ Abstract

Background: Non-nutritive sweeteners are commonly used as sugar substitutes to provide sweet taste without calories. However, there is controversy regarding their safety and potential health effects. This study evaluated the effects of commercial non-nutritive sweeteners including sucralose, aspartame, saccharin, acesulfame-K and stevia on oxidant/antioxidant balance in albino rats. **Methods:** Six groups of rats were administered optimized doses of sweeteners for 8 weeks. Oxidative stress was assessed by measuring malondialdehyde (MDA) and total antioxidant status (TAS) before and after treatment. **Results:** Aspartame and stevia significantly increased TAS whereas sucralose and acesulfame-K decreased it. All sweeteners except stevia significantly increased MDA levels. **Conclusion:** The results indicate that long-term intake of commercial non-nutritive sweeteners disrupt redox homeostasis in rats. However, stevia may have relatively less adverse effects. Further research is warranted to fully elucidate the mechanisms and health implications.

Keywords: Non-nutritive Sweeteners, Artificial Sweeteners, Oxidative Stress, Antioxidant Status, Rat Model.

1. Introduction

Artificial sweeteners, also known as intense sweeteners, are additives that provide little or no calories. They are widely used as low-calorie alternatives to sugar in foods, beverages and tabletop sweeteners. The intake of non-nutritive sweeteners has increased dramatically in recent years, driven by rising rates of obesity and diabetes as well as recommendations to limit sugar intake [1-5]. The five non-nutritive sweeteners that have been approved by the U.S. Food and Drug Administration (FDA) include sucralose, aspartame, saccharin, acesulfame-K and steviol glycosides (FDA, 2018) [6-8].

The chlorination of sucrose turn sucrose into sucralose, the latter is about 600 times sweeter than former [9-12]. Compared to sucrose, aspartame is nearly 200 times sweeter than sucrose [13-15]. Compared to sucrose, saccharin is nearly 200-700 times sweeter than sucrose [16, 17]. Compared to sucrose, Acesulfame-K is nearly 200 times sweeter than sucrose [18, 19]. Compared to sucrose, extracted from the stevia rebaudiana plant is nearly 300 times sweeter than sucrose [20, 21].

These non-nutritive sweeteners are widely marketed as healthy sugar alternatives that can help with weight management and blood glucose control without compromising taste. However, there is controversy concerning the safety and potential health impacts of their long-term consumption. Studies have linked intake of artificial sweeteners to adverse cardiometabolic outcomes including glucose intolerance, insulin resistance, overweight/obesity, stroke and hypertension [22, 23]. The mechanisms by which non-nutritive sweeteners may cause harm are not fully understood but may involve effects on gut microbiota, appetite signalling and metabolic regulation [24-26].

One proposed mechanism is through disruption of oxidant/antioxidant balance and induction of oxidative stress [27]. Oxidative stress ensues when there is an dysregulation between synthesis of reactive oxygen species (ROS) and the endogenous scavengers of free radical. ROS are formed during normal cellular metabolism but at high levels can damage DNA, proteins and lipids. The body has endogenous antioxidant systems including superoxide dismutase, glutathione peroxidase and catalase that neutralize ROS and prevent oxidative damage [28-30].

However, excessive ROS generation can overwhelm natural antioxidant capacity leading to oxidative stress. Chronic oxidative stress has been implicated in pathogenesis of obesity, insulin resistance, diabetes, cardiovascular and neurodegenerative diseases [31]. Non-nutritive sweeteners could potentially induce oxidative stress by altering metabolic pathways, depleting antioxidants and/or impairing mitochondrial function [32].

Malondialdehyde (MDA) is a commonly used test of lipid peroxidation and oxidative stress. Plasma MDA levels have been found to increase in consumers of saccharin[33] and aspartame [27]. Total antioxidant status (TAS) provides an indication of the body's capacity

 DOI 10.1900/RDS.2024.20.6

to counteract ROS. Studies report that sucralose [32], aspartame [33] and saccharin [34] reduce TAS in rats. However, there is limited research comprehensively evaluating and comparing the effects of the major commercial non-nutritive sweeteners on oxidant/antioxidant balance.

Therefore, the study under investigation aimed to determine the impacts of long term intake of sucralose, aspartame, saccharin, acesulfame-K and stevia on oxidative stress parameters including MDA and TAS in albino rats. The findings will provide insight into the mechanisms and safety of non-nutritive sweeteners with regards to redox homeostasis and health.

2. Materials and Methods

Experimental animals: Albino rats: Six groups of white Albino rats were obtained for this research. The rats were housed at 25±3°C with 12 hour light/dark cycles and provided with standard laboratory food and tap water [35-38].

To start the experiment, the rats were randomly divided into the following six groups of 10 rats each:

Group 1: Received regular diet and water (normal control)

Group 2: Received 10% sucrose solution

Group 3: Received optimized dose of aspartame (250mg/kg/day)

Group 4: Received optimized dose of saccharin

Group 5: Received optimized dose of acesulfame-K (250mg/kg/day)

Group 6: Received 4% stevia solution

The sucrose group served as a positive nutritive sweetener control. The optimized doses of the non-nutritive sweeteners were selected based on prior studies demonstrating metabolic effects in rats. The sweetener solutions were prepared fresh daily and administered to the rats by oral gavage for 8 consecutive weeks. Throughout the 8 weeks treatment timeframe, the rats were given unlimited access to standard lab chow and water from the tap. The weights of the rats were checked weekly over the full 8 weeks.

Following the completion of the 8 weeks dosing timeframe, the rats underwent a fasting period overnight and were then put under anesthesia using ether. Blood was collected from the rats via cardiac puncture into tubes containing heparin. The blood samples were centrifuged to isolate the plasma, which was then stored at -80°C until the time of analysis.

2.1. Biochemical Analysis

An ELISA kit (Cat. No. MBS16000693, MyBioSource) was used to measure total antioxidant status (TAS) in the rats. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid (TBA) method [39]. After the 8-week treatment period, blood samples were collected and serum was analyzed for TAS and MDA levels.

Statistical analysis: Data expressed as mean±SD. One-way analysis of variance was used to compare between means of groups and series of paired t-test were used to compared before and after intervention. Duncan's test used to test the significant group. P<0.05 was considered significance.

3. Results

Effects on Plasma Total Antioxidant Status: The effects of chronic administration of nutritive and non-nutritive sweeteners on plasma total antioxidant status (TAS) in albino rats are presented in Figure 1. At baseline, there were no significant differences in mean TAS levels across the groups, ranging from 0.14 – 0.15 mmol/L. After 8 weeks, plasma TAS remained unchanged in the normal control group. However, the sucrose control exhibited a significant decrease in TAS from 0.15 ± 0.01 to 0.12 ± 0.007 mmol/L (P<0.05).

Among the non-nutritive sweetener groups, aspartame and stevia significantly increased TAS levels by 55% (P<0.05) and 13% (P<0.05) respectively compared to baseline. In contrast, sucralose and acesulfame-K induced significant reductions in TAS by 22% (P<0.05) and 14% (P<0.05) respectively. When compared between groups, aspartame and stevia supplementation resulted in significantly higher post-treatment TAS versus all other groups (P<0.05).

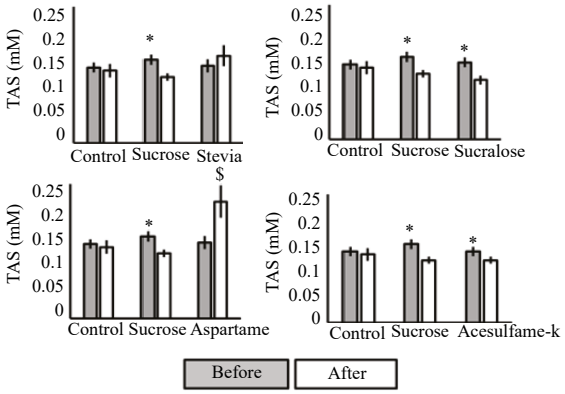

Figure 1: Plasma Total Antioxidant Status (TAS) of Albino Rats Treated with Nutritive and Non-nutritive Sweeteners for 8 Weeks. Values Represent Mean ± SD (n=10 Per Group). * P<0.05 Compared to Baseline; $ P<0.05 Compared to Normal Control.

Effects on Plasma Malondialdehyde: The impacts of chronic sweetener consumption on plasma malondialdehyde (MDA) levels in rats are shown in Figure 2. Baseline MDA concentrations were similar across groups, ranging from 13.1 – 13.6 µmol/L. After 8 weeks, there were no significant changes in the normal control group. Sucrose supplementation elicited a substantial 40% increase in MDA versus baseline (P<0.05). All non-nutritive sweeteners also provoked significant elevations in plasma MDA relative to pretreatment values (P<0.05).

However, stevia exhibited the lowest rise in MDA of only 16% above baseline while aspartame had the highest effect with a 37% increment. Sucralose and acesulfame-K increased MDA by 29% and 30%

respectively. Comparison between groups revealed that aspartame produced the highest post-treatment MDA out of all sweeteners (P<0.05). Stevia maintained the lowest MDA level which was comparable to normal control and significantly lower than sucrose, sucralose and acesulfame-K (P<0.05).

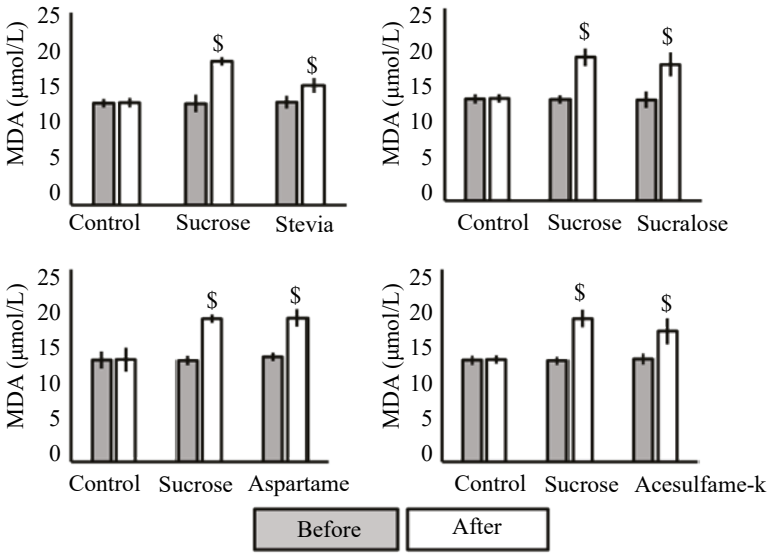

Figure 2: Plasma Malondialdehyde (MDA) Levels of Albino Rats Treated with Nutritive and Non-nutritive Sweeteners for 8 Weeks. Values Represent Mean ± SD (n=10 Per Group). $ P<0.05 Compared to Baseline.

Comparison of Sweetener Effects on Oxidative Stress Markers: To directly compare the effects of the nutritive and non-nutritive sweeteners, the post-treatment values of TAS and MDA were plotted together as shown in Figures 3 and 4 respectively. For TAS, aspartame and stevia supplementation resulted in significantly higher levels compared to all other groups (P<0.05). Sucralose, acesulfame-K and sucrose did not differ from each other but showed markedly lower TAS than the aspartame and stevia groups (P<0.05).

Regarding MDA, stevia maintained the lowest concentration which was significantly different from the other sweetener treatments (P<0.05). Aspartame exhibited the highest MDA out of all groups (P<0.05) while sucralose, acesulfame-K and sucrose showed intermediate elevations that were comparable to each other.

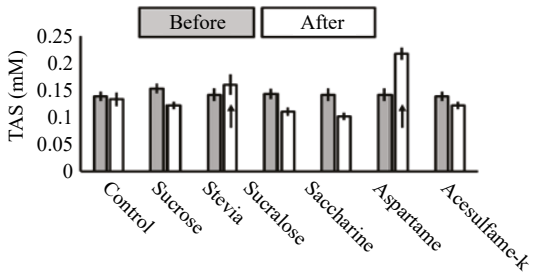

Figure 3: Comparison of Plasma Total Antioxidant Status (TAS) in Albino Rats Treated with Nutritive and Non-nutritive Sweeteners for 8 Weeks. Values Represent Mean ± SD (n=10 Per Group). * P<0.05 Compared to All Other Groups.

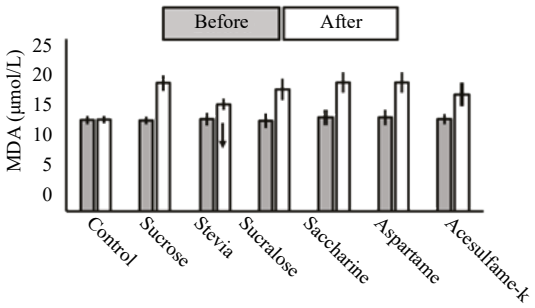

Figure 4: Comparison of Plasma Malondialdehyde (MDA) Levels in Albino Rats Treated with Nutritive and Non-nutritive Sweeteners for 8 Weeks. Values Represent Mean ± SD (n=10 Per Group). * P<0.05 Compared to All Other Groups.

4. Discussion

Non-nutritive sweeteners are widely used as zero- or low-calorie sugar substitutes in foods, beverages and tabletop sweeteners. They provide sweet taste without the calories of nutritive sugars like sucrose. Although marketed as healthy alternatives, there is controversy regarding the safety and metabolic effects of chronic consumption of non-nutritive sweeteners. One proposed mechanism by which artificial sweeteners may exert adverse effects is through disruption of oxidant/antioxidant homeostasis and induction of oxidative stress. This study compared the impacts of the five major FDA-approved non-nutritive sweeteners namely sucralose, aspartame, saccharin, acesulfame-K and stevia on oxidative stress parameters in albino rats.

The results demonstrated that sucralose, aspartame, saccharin and acesulfame-K significantly increased

lipid peroxidation marker MDA and reduced plasma total antioxidant status, indicating induction of systemic oxidative stress. These findings concur with earlier reports of increased oxidative damage and compromised antioxidant defences in consumers of sucralose [32], aspartame [33], saccharin [40] and acesulfame-K [34]. Several possible mechanisms could account for the pro-oxidant effects observed. These artificial sweeteners or their metabolites may directly generate free radicals and reactive species [27].

Sucralose has been found to suppress antioxidant enzymes like superoxide dismutase and glutathione peroxidase which allow unchecked ROS accumulation [32]. Aspartame and its metabolite methanol can reduce glutathione pools thereby limiting antioxidant capacity [33]. Saccharin impairs liver and kidney mitochondrial function resulting in increased ROS generation [41]. Acesulfame-K disturbs the Nrf2 antioxidant pathway enabling oxidative damage [18].

In contrast to the other sweeteners, stevia supplementation significantly enhanced TAS and minimized elevation of MDA in this study. Steviol glycosides in stevia exhibit free radical scavenging activity and can boost cellular antioxidant defenses through Nrf2 activation [21]. The stevia dosage used in this study was well below the ADI and approximated normal human intake levels. Safety studies have not found adverse effects of stevia even at much higher doses [20]. Therefore, stevia appears to have relatively less detrimental impacts on redox balance compared to synthetic non-nutritive sweeteners.

Oxidative stress is implicated in the pathogenesis of many chronic diseases including diabetes, cardiovascular disorders, cancer and neurological conditions [28]. The present findings indicate that frequently consuming sucralose, aspartame, saccharin or acesulfame-K even at ADI levels may perpetuate oxidative stress and inflammation. This could potentially contribute to development of obesity, insulin resistance, hypertension, stroke and other cardiometabolic abnormalities that have been associated with intake of artificial sweeteners [22, 23]. On the other hand, stevia may have therapeutic antioxidant properties and attenuate redox-related pathology. Further research is required to fully elucidate the clinical ramifications of long-term artificial sweetener consumption.

This study had some limitations that should be acknowledged. Firstly, it was conducted in albino rats which may not fully reflect human physiology and metabolism. Secondly, only two markers of oxidative stress were assessed. Evaluation of additional parameters such as antioxidant enzymes, glutathione, protein carbonyls and DNA damage could provide further insight. Thirdly, the specific cellular, molecular and signalling mechanisms involved remain to be characterized. Further studies investigating dose-response effects, impact of sweetener combinations and underlying pathways would be beneficial. Fourthly, only male rats were used so potential sex differences could not be determined. Finally, implications for development of chronic disease were not evaluated directly. Long-term animal studies monitoring disease outcomes would offer additional evidence regarding health risks.

5. Conclusion

In conclusion, this study indicates that chronic intake of commercial non-nutritive sweeteners including sucralose, aspartame, saccharin and acesulfame-K disrupts oxidant/antioxidant balance and induces oxidative stress in albino rats, while stevia has relatively less adverse effects on redox homeostasis. These findings add to the growing body of preclinical evidence suggesting that frequent consumption of synthetic artificial sweeteners may have detrimental health impacts. More research is warranted to fully characterize the mechanisms involved and to clarify the relationships between non-nutritive sweetener intake and risk of chronic diseases in humans. Nonetheless, the results suggest stevia may be a safer alternative and highlight the need for a precautionary approach regarding chronic use of sucralose, aspartame, saccharin and acesulfame-K pending more conclusive data on their long-term safety.

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