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Combination of Aspartame and Acesulfame Potassium Does not Alter Blood Glucose, Lipids and Cytokines of Female Rats

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ABSTRACT: The use of combination of artificial sweeteners, most importantly aspartame (E951) and acesulfame potassium (E950) in foods, beverages and other products is on the increase despite reports on their side effects. This study investigated the effects of combination of tolerable daily dose of both aspartame and acesulfame-k on blood glucose, lipids and cytokines of female Wistar rats. Twenty-five female Wister rats weighing 90.7±8.1 g were randomly assigned into five groups. Groups 1 (control group) were administered 0.5 mL distilled water, Group 2; 0.5 mL mixture of 50 mg/kg b.w./day aspartame and 15 mg/kg b.w./day of acesulfame-k, Group 3; 0.5 mL of 15 mg/kg b.w./day of acesulfame-k, Group 4; 0.5 mL of 50 mg/kg b.w./day of aspartame and Group 5; 0.5 mL 330 mg/kg b.w./day of sucrose, all for twenty eight days. The biochemical parameters blood glucose, lipids and cytokines were assayed. Results obtained showed that blood glucose level, lipids and cytokines of rats exposed to the mixture of acesulfame-k and aspartame were not significantly altered compared to the control rats. In conclusion, administration of combination of acesulfame-k and aspartame to the female rats at the tolerable daily intake level did not disturb the blood glucose, lipid and cytokine status.

Keywords: Acesulfame-K, Aspartame, Blood glucose, Lipids, Cytokines

Introduction

Sugars have been employed as sweeteners to enhance the flavor and caloric content of meals over time (Saraiva *et al*., 2020). But the rising rate of obesity attributed to this use has led to a considerable rise in the demand for alternative low caloric diets (Mora and Dando, 2021). This has caused emergence of a variety of artificial sweeteners to appear in the food and drink industry (Wilk *et al*, 2022). Artificial sweeteners like aspartame and acesulfame-k are cheaper and sweeter which contain lower caloric value preferably to traditional sugars thus are better alternatives (Souza *et al*., 2022; Wilk *et al*., 2022). Food industries combine these additives in drinks since a good amount of them possess varying solubility, duration, stability, and after-taste effect (Eggleston, 2019).

Acesulfame potassium is a non-nutritive sweetener 200 times sweeter than sucrose (Helal *et al*., 2019). It is used as a sweetener in variety of foods, including baked, canned and desserts foods. It is not retained or processed by the body (Helal *et al*., 2019). Aspartame is a sweetener that has been utilized for years as a means of reducing one's consumption of sugars while still allowing them to experience sweetness (Newbould *et al*., 2021). Considering that aspartame is 200 times sweeter than sugar, very little of the sweetener is required to compete with the sweetness of sugar (Khamise *et al.*, 2020). Both aspartame and acesulfame-k have tolerable daily intake level of 50 mg/kg b.w./day and 15 mg/kg b.w./day respectively (Garavaglia *et al.*, 2018).

Serious health concerns have been linked to the consumption of the sweeteners separately in processed foods. These claims include acesulfame potassium contains the carcinogenic methylene chloride. Methylene chloride can cause headaches, depression, nausea, mental confusion, liver effects, kidney effects, and cancer (Helal *et al.,* 2019). Long-term consumption of aspartame leads to hepatocellular injury and alters the redox status of the liver with deleterious effects on liver antioxidant status (AL-Hadrawy, 2022). A review of animal research indicated that massive doses of artificial sweeteners cause excess weight through hypoglycemia and increase feed intake (Mora and Dando, 2021). Other notable issue linked with these sweetening agents include; liver cancer (Jones *et al* 2022), hematopoietic cancer (Debras *et al*., 2022), type II diabetes (Nadipelly *et al*., 2017), hypertension (Liu *et al.,* 2022), and coronary diseases (Pase *et al*., 2017). Given this background, the consumption of sugar substitutes as artificial additive has become questionable and warrant further assessment.

The adverse effects of sweeteners are well publicized. This wealth of reports addressed the use of single sweeteners or combination. Despite the side effects reported for the use of each of aspartame and acesulfame potassium, these two are continuously found in foods and drinks like carbonated drinks, beverages, cereals, sugar-free sweets and others. Combination of these two sweeteners would probably be accompanied with greater side effects reported for each but there is limiting information on the safety/risks of use of combined aspartame and acesulfame potassium on the blood glucose, lipid and cytokine status. So, this study was attempted to bridge the gap of insufficient information on the safety of the combination in the area.

Materials and methods

Chemicals and reagents: Aspartame and Acesulfame-K were obtained from Vitasweet®, Co., Ltd. China, manufactured in Shanghai, China. Other materials like glucometer/stripes were obtained from ON CALL PLUS, India and assay kits for lipids and cytokines were obtained from Abcam, Cambridge and Biogene Lab in Delhi, respectively.

Experimental animals: Twenty five female wistar rats with an average weight range of 90.7 ± 8.1 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. All the rats were housed in plastic cages placed in a well-ventilated Animal House. The rats were allowed free access to feed and tap water, and were handled strictly according to the instructions guidelines on Care and Use of Laboratory Animals (Okoduwa *et al.,* 2017).

Animal grouping and treatment: Twenty five female rats were divided into five groups of 5 rats each and were given the following treatments.

Group 1: Rats were given 0.5mL of distilled water

Group 2: Rats were given 0.5mL of combined 50 mg/kg b.w./day aspartame and 15 mg/kg of acesulfame-k solution.

Group 3: Rats were given 0.5mL of 15 mg/kg b.w./day acesulfame-k solution.

Group 4: Rats were given 0.5ml of 50 mg/kg b.w./day aspartame solution.

Group 5: Rats were given 0.5ml of 330 mg/kg b.w./day sucrose solution.

The doses were administered orally with the aid of a plastic oral-pharyngeal cannula once daily for 28 days. The rats' body weights were recorded at the beginning and on weekly basis throughout the period of experiment.

Sacrifice of rats and collection of blood: Twenty-four hours after the last dose, the rats were weighed and then anesthetized in diethyl ether fumes when they became unconscious the jugular veins were cut using a sharp razor blade and the blood samples were collected into clean, test tubes free of anticoagulants.

Preparation of serum: The blood samples collected were then centrifuged for 15 min at 503 x *g.* The serum was separated with a pipette and used for the biochemical assay i.e. lipid and cytokine status.

Biochemical examination: Blood glucose was determined by glucometer using whole blood while the concentration of insulin in the serum was determined via the tube-based serum enzyme immunoassay (EIA) method. Total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were estimated in the serum by enzyme colorimetric method according to Fredrickson *et al.* (1967), Hainline *et al.* (1980), Albers *et al.* (1978) and Friedwald *et al.* (1972) respectively. Pro-inflammatory cytokines- Interleukin 1b and 6 were estimated in the serum by ELISA kit according to Abcam (2017).

Data analysis: Results were presented as mean ± standard error of mean (S.E.M) of 5 replicates, and were subjected to one way Analysis of Variance (ANOVA). The results were considered statistically significant at $p < 0.05$ using GraphPad Prism version 6.01 (GraphPad Software, Inc., San Diego, California, United States).

Results

The blood glucose level of the rats treated with combination of aspartame and acesulfame-k was not significantly ($p > 0.05$) altered when compared to the control rats (Table 1). In the same vein, the insulin level of the rats was not significantly affected. The lipid profile of the rats administered with combination of aspartame and acesulfame-k was not significantly ($p > 0.05$) changed when compared to the control rats (Table 2). Interleukin 1b and 6 of the rats administered with combination of aspartame and acesulfame-k was not significantly ($p > 0.05$) affected when compared to the control rats (Table 3).

Table 1: Blood glucose level of female rats exposed to combination of aspartame and acesulfame-k

Blood glucose level (mmol/dl)	Control	Aspartame+ Acesulfame-k	15mg/kg B.wt Acesulfame-k	50mg/kg B.wt Aspartame	330mg/kg B.wt Sucrose
Day 14	4.20 ± 0.35[a]	4.53 ± 0.47 [a]	4.43 ± 0.27[a]	5.03 ± 0.12 [a]	5.10 ± 0.57 [a]
Day 17	4.17 ± 0.15 [a]	4.57 ± 0.45 [a]	4.53 ± 0.18 [a]	4.73 ± 0.38 [a]	4.80 ± 0.70 [a]
Day 20	4.40 ± 0.12 [a]	4.63 ± 0.84 [a]	4.63 ± 0.61 [a]	5.07 ± 0.17 [a]	5.43 ± 1.13 [a]
Day 23	3.87 ± 0.12 [a]	3.93 ± 0.44 [a]	4.17 ± 0.35 [a]	4.50 ± 0.57 [a]	5.07 ± 1.22[a]
Day 28	3.73 ± 0.03 [a]	3.97 ± 0.81 [a]	3.97 ± 0.93 [a]	3.97 ± 0.63 [a]	4.53 ± 0.78 [a]

Values are mean of 5 replicates ± SEM, values with the same superscript across the rows for each parameter are not significantly different at $p > 0.05$

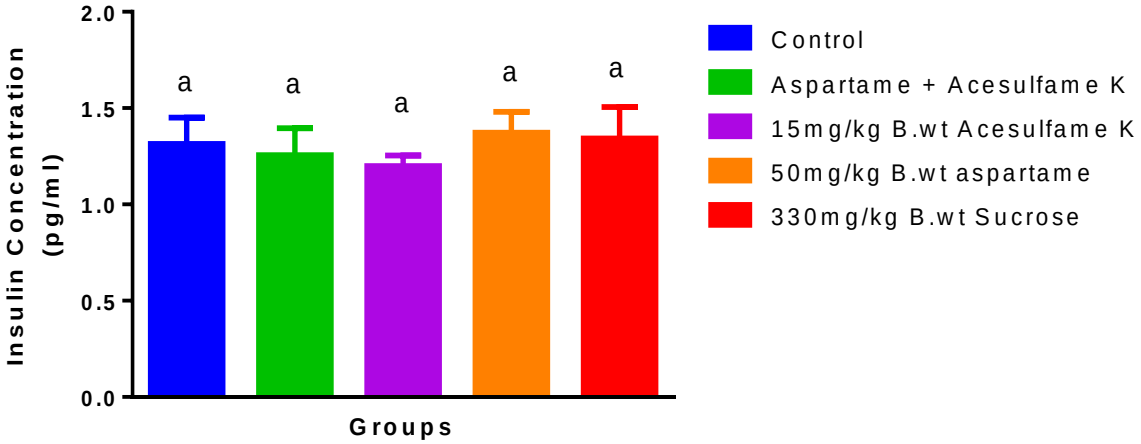

Figure 1: Insulin concentration in serum of female rats treated with combination of acesulfame potassium and aspartame. Values are presented as mean ± S.E.M of five replicates. Values having same alphabet are not significantly different at ($p > 0.05$)

Table 2: Lipid profile of female rats administered combination of aspartame and acesulfame-k

Parameter (mg/dl)	Control	Aspartsame+ Acesulfame-k	15mg/kg B.wt Acesulfame-k	50mg/kg B.wt Aspartame	330mg/kg B.wt Sucrose
Cholesterol	26.28 ± 2.35[a]	24.34 ± 1.12[a]	25.04 ± 1.71[a]	24.29 ± 2.76[a]	25.62 ± 1.76 [a]
Triglycerides	9.98 ± 0.53[a]	10.65 ± 0.21[a]	9.59 ± 0.57 [a]	9.85 ± 0.55 [a]	10.22 ± 0.49 [a]
High Density Lipoprotein-CH	10.29 ± 0.71 [a]	11.62 ± 0.26 [a]	11.93 ± 0.40 [a]	11.95 ± 0.46 [a]	10.92 ± 0.42 [a]
Low Density Lipoprotein-CH	13.31 ± 2.92 [a]	12.69 ± 0.25 [a]	13.10 ± 0.41 [a]	13.00 ± 0.07 [a]	13.51 ± 0.91[a]

Values are mean of 5 replicates ± SEM, values with the same superscript across the rows are not significantly different at $p > 0.05$.

Table 3: Cytokines of female rats exposed to combination of aspartame and acesulfame-k

Parameters (ng/ml)	Control	Aspartame+ Acesulfame-k	15mg/kg B.wt Acesulfame-k	50mg/kg B.wt Aspartame	330mg/kg B.wt Sucrose
Interleukin 1β	14.61 ± 0.86[a]	15.01 ± 2.24 [a]	15.65 ± 2.38 [a]	13.34 ± 1.14 [a]	15.07 ± 2.08 [a]
Interleukin 6	1.56 ± 0.10 [a]	1.42 ± 0.09 [a]	1.42 ± 0.10 [a]	1.71± 0.05 [a]	1.45 ± 0.71 [a]

Values are mean of 5 replicates ± SEM, values with the same superscript across the row are not significantly different at $p > 0.05$

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Discussion

Artificial sweeteners are known as low calorie and sugar substitute compounds (Ajami *et al.,* 2020; Hess *et al.,* 2018). Because of the concern that sugars increase the risk of obesity, diabetes and cardiovascular diseases, these artificial sweeteners have been used to replace sugars (Wilk *et al.,* 2022). Accumulation of artificial sweeteners is associated with elevated glucose in blood which results to lipid accumulation in organs and tissues (Shi *et al*, 2019).

In this study, the blood glucose level was not altered when compared to the control rats. This is at variance with report of Saraswathy and Mishra (2018) that there was increased blood glucose level in rats that received acesulfame-k and aspartame. The insulin level of the rats was not disturbed, this is an indication of homeostasis in glucose metabolism. Therefore combination of aspartame and acesulfame-k at tolerable daily intake might not lead to hyperglycemia which is a major risk factor for diabetes.

Dyslipidemia is commonly characterized by increased levels of serum total cholesterol (TC), low density lipoprotein (LDL), and triglyceride (TG) with a concomitant decrease in the concentration of serum high density lipoprotein (HDL) (Asbaghi *et al.,* 2021). Study conducted by Santos *et al (*2018) claimed that aspartame consumption was not associated with alterations on TC and TG but only on HDL concentration. Movahedian *et al* (2021) revealed that artificial sweeteners intake may not affect serum levels of TG, TC, LDL, and HDL.

The lipid profile of the rats in this study was not changed. This agreed with these claims and opposed report of Helal *et al.* (2019) that there was elevated lipid profile in rats that received acesulfame-k and aspartame. This suggests that combination of aspartame and acesulfame-k at tolerable daily intake might not cause dyslipidemia which is a major factor for atherosclerosis.

Excessive sucrose consumption had been stated to elevate pro-inflammatory cytokines in human blood (Fulton *et al.,* 2022). The levels of pro-inflammatory cytokines; interleukin 1b and 6 in the rats of this study was not significantly altered signifies that combination of aspartame and acesulfame-k at the tolerable daily intake does not cause inflammation nor the activation of immune response. Unchanged blood glucose and cytokines agreed with Subali *et al* (2017) which suggested that pro-inflammatory cytokines are not elevated at normal glucose level concentration.

Conclusion

This study showed that administration of combination of acesulfame-k and aspartame to female rats at the tolerable daily dose (15 mg/kg b.w./day of acesulfame-k, 50 mg/kg b.w./day of aspartame) did not disturb the blood glucose, lipid and cytokine status.

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