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Comparative Study between Artificial Sweeteners Such as Aspartame and Neotame on Neurobehavioral and Some Haematological Parameters in Male Rats

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Abstract

Recent studies have suggested potential health risks associated with the consumption of artificial sweeteners. This study investigates the effects of the artificial sweeteners aspartame and neotame on neurobehavioral performance and selected hematological parameters in male rats. Twenty-four male albino rats, weighing 200 to 240 g, were divided into three groups: a control group receiving tap water, a group receiving neotame (250 mg/kg/day), and a group receiving aspartame (500 mg/kg/day). The study was conducted over eight weeks under controlled conditions. Neurobehavioral assessments included the open field test, swimming rank test, and Y-maze test. Hematological parameters were analyzed using an automated blood analyzer after the experimental period. The findings indicated that both aspartame (ASP) and neotame (NEO) significantly affected neurobehavioral parameters in male rats. The open field test results demonstrated a marked reduction in locomotor activity in both treatment groups compared to controls ($p < 0.05$). Similarly, the swimming rank test revealed diminished swimming capabilities in the ASP and NEO groups, suggesting potential neurotoxic effects. Hematological analyses showed a significant increase in red and white blood cell counts in both treatment groups, while hemoglobin and hematocrit levels decreased compared to controls ($p < 0.05$). The study concludes that chronic exposure to artificial sweeteners such as aspartame and neotame can adversely affect neurobehavioral and hematological parameters in male rats. These findings underscore the importance of further research to clarify the long-term health effects of artificial sweeteners in both animal models and humans.

1. Introduction

Consuming artificial sweeteners has been linked to significant health hazards, according to recent studies. Aspartame (ASP) is an artificial, non-saccharide sweetener, L-aspertyl-L phenylalanine methyl ester that is a methyl ester of the dipeptide of the amino acids aspartic acid and phenylalanine. Neotame (NEO) has been developed as a sweetener with a high degree of sweetness and is obtained by N-alkylating aspartame. Global

market reports on artificial sweeteners indicate that the US Food and Drug Administration (FDA) has authorized saccharin, sucralose, aspartame, neotame, acesulfame potassium, and cyclamate as safe and commonly used artificial sweeteners. Artificial sweeteners are chemically synthesized compounds are used to sweeten foods and drinks in place of sucrose. Much smaller amounts of artificial sweeteners are required to achieve the same level of sweetness because they are many times sweeter than table sugar [1]. The US Food and Drug Administration (FDA) has authorized aspartame (L-aspartyl L-phenylalanine methyl ester), a non-nutritive sweetener, for use as an artificial sweetener and in carbonated soft drinks at a maximum dosage of 50 mg/kg body weight [2]. One of the most popular nonnutritive, strong sweeteners in the world, Aspartame has been approved by various food regulatory agencies for use as a tabletop sweetener and in a variety of foods, such as desserts, yoghurt, carbonated soft drinks, weight-control products, and confections [3]. Numerous health problems, including neurological and behavioral symptoms (such as convulsions and hyperactivity), allergic-type reactions, and cancer (brain and breast), were allegedly linked to dietary exposure to aspartame during the post market surveillance period such as other conditions, including multiple sclerosis, lupus, and Alzheimer's disease, were also attributed to aspartame exposure [4]. These claims were fueled by concerns about the integrity of the studies used to support approval as well as alleged biases in the original US approval process. Both individual studies and comprehensive toxicological evaluations that looked into these allegations found no evidence of a connection to aspartame exposure [5].

A synthetic dipeptide sweetener, aspartame is more than 200 times sweeter than sucrose. This is a peculiar characteristic for a peptide, which typically has a cheesy, savory, or bitter taste, like the isomeric form β-aspartame. Two carboxylic groups make up aspartic acid, and early chemical production methods resulted in enormous amounts of β-aspartame, a byproduct that is really bitter rather than sweet [6]. Aspartame and neotame are closely linked as well. The sole structural variation is that neotame has a -NH-alkyl group in place of the -NH3+ group found in aspartame [7]. Being 7000–13,000 times sweeter than sucrose, neotame is the strongest sweetener currently on the market. Neotame, which resembles aspartame and is a peptide, is about as stable as aspartame, with the potential to be somewhat more stable when heated [8]. Neotame has been reported to enhance other flavors and provide a clean, sweet taste free of overtones of bitterness or metal [9].

Data from three studies of four cohorts that examined associations with artificially sweetened beverages during times when such beverages primarily included aspartame were used to support the recent discovery of a potential link between aspartame and liver cancer in humans [10]. While the study identified an association among individuals with diabetes, the second study indicated a relationship with liver cancer risk in the entire population [11]. There was no correlation with liver cancer in the third [12]. A fourth study also examined the association between artificially sweetened beverages and liver cancer risk [13]. Aspartame is the precursor to both neotame and advantame. These artificial sweeteners have been linked [14]. This study aims to examine the effects of artificial sweeteners such as aspartame (ASP) and neotame (NEO) on the neurobehavioral and hematologic parameters of rats.

2. Experimental Procedure

2.1. Ethics

The study was conducted in accordance with the ethical guidelines approved by the Ethics and Scientific Committee at the College of Veterinary Medicine, Al-Qasim Green University (Approval No. 5304, dated 03/07/2024).

2.2. Study Design

All chemicals used in this study, including pure ASP and NEO, were of analytical grade and procured from (Sinochem/ China) and medical services. In this study, Twenty-four adult male albino rats, weighing 200–240 g were housed in three stainless steel cages under controlled conditions (12-hour light/dark cycle; 25 ± 3°C).The local ethics committee gave its approval to the experimental procedures (222-2024). The experimental procedures were conducted in accordance with the guidelines of the U.S. National Institutes of Health (NIH) for the Care and Use of Laboratory Animals. This study included three groups of rats. The control group consisted of eight rats that were given tap water. The drinking water for the other two groups contained NEO (500 mg/kg/day, n = 8) and ASP (250 mg/kg/day, n = 8) [15]. After eight weeks, a passive avoidance test was used to

evaluate how sweeteners affected memory and learning. Following euthanasia, hematological and neurobehavioral assessments were performed.

2.3. Neurobehavioral Study

2.3.1. Open Field Tests

A popular method for evaluating a compound's sedative, poisonous, or stimulating properties is the open field test. Therefore, in addition to measuring movement, the open field test assesses other aspects of behavior. As a result, the test serves a variety of purposes and is a part of nearly all comprehensive analyses of rodent behavior. This test assesses the animal's general locomotor activity, rearing, exploration (the forward and backward movement of all four legs), and frequency of urination and faces. For a period of three minutes, the rats were positioned in the middle of an open field device, and their movements were monitored. We kept track of the number of squares traversed, as well as fecal and urine samples and incidences of rearing. After every trial, we cleaned the arena completely. The arena is made up of a 100 × 100 cm2 hardwood open box. It is divided into sixteen equal squares, each of which is 20 by 20 cm^2 and suitable for adult rats [16].

2.3.2. Swimming Rank Test

It is a 30-cm tall glass pool filled with heated water (30 °C). This test assesses brain function integration by monitoring each animal swimming for 5-10 seconds (For adult rats, the pool's dimensions were 70 x 40 x 40 cm) containing water heated to 30 °C, and the evaluation was carried out through grading [17].

2.3.3. Y-Maze Test Alternate Arm/3min

The Y-maze in this investigation was built in according to the guidelines provided by [18]. The hippocampus is not essential for object-recognition function in object-recognition memory, the arm-recognition component of the Y maze has been used in its place. The Y-maze consists of three identical arms, each measuring 50 cm in length, and 16 cm in width, and 32 cm in height on the sides. Two sets of infrared photocells were attached to each arm, with a distance of 21.

2.4. Hematologic Study

An automatic blood analyser for rats manufactured by Mindary Company, China, was used to examine blood samples. Hematological analysis was performed on whole blood samples treated with EDTA were measured using standard techniques with an automated hematology analyzer, hematological parameters such as the quantity of red blood cells (RBC), white blood cells (WBC), platelets (PLT), hemoglobin (Hb) level, and hematocrit (Hct) values were measured in whole blood.

2.5. Statistical Analysis

The statistical program Graphpad Prism for Windows, version 6.0 was used for statistical analysis. The means ± S.D were used to present the data. Tukey's post-hoc test was used for multiple comparisons between treatments. After a one-way analysis of variance (ANOVA) was used to statistically evaluate the effects of the treatments. Statistical significance was determined at the $p < 0.05$ level.

3. Results

3.1. The Open Field Test

3.1.1. Number of Square Crossed in Arena/3min

Table (1) showed the open field test measured the number of squares crossed by the four legs/3min. The administration of 500 mg/ kg.b.w of ASP and NEO significantly reduced ($p < 0.05$) the number of squares crossed by four legs/3 minutes in both treatment groups of the study as compared to x=zero-time measurements, according to the results shown in Table (1). By the end of the eight-week experiment, the results were statistically significant compared to control group (p-value < 0.05).

Table (1): The open field test, number of square crossed in arena/3min.

Groups N=8	Zero time (mean± S.E)	8 weeks (mean± S.E)
Control	19±1.13Aa	22.03±1.33Aa
ASP 500mg/kg	20±2.01Aa	11.01±2.04Bb
NEO 500mg/kg	21±1.19Aa	13±1.94Bb
LSD	5.28	

Different capital letters indicate statistical differences among groups while lowercase (or small letters) letters indicate statistical differences between time points ($p < 0.05$)

Figure (1) represented the bar chart showing the 8-week mean values for each group with standard error bars. The dashed gray lines consider the Least Significant Difference (LSD) threshold for comparing the Control group to others. ASP and NEO means fall below the lower LSD threshold, indicating significant differences from Control. ASP and NEO are not significantly different from each other, as their means are within the LSD range.

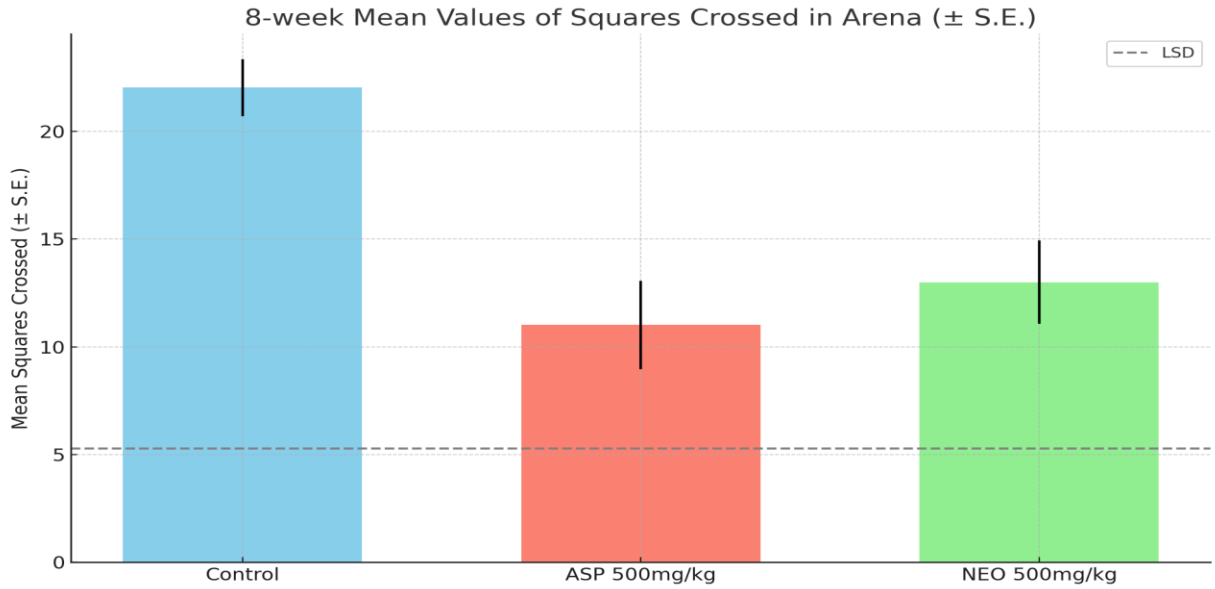

Figure (1): 8-Week Treatment Group Means with SE and LSD Threshold.

3.1.2. Number of Bolus Faces/3min

Table (2) presented that there was no statistically significant difference ($p < 0.05$) in the number of faecal boluses every 3 minutes among the groups.

Table (2): The open field test, measuring the number of boluses faces/ 3 minutes.

Groups N=8	Zero time (mean± S.E)	8 weeks (mean± S.E)
Control	2.91±0.77Aa	2.05±0.94Ab
Asp 500mg	2.70±0.64Aa	1.66±0.67Bb
Neo 500mg	3.38±0.51Aa	1.13±0.83Bb
LSD	1.17	

Different Capital letters denote among groups statistical differences where small letters denote between periods statistical differences at ($p < 0.05$)

3.1.3. Number of Urination/3min

As shown in Table (3), there was no discernible variation in the frequency of urine across the experimental groups during the open field test at most time periods ($p>0.05$). However, the ASP and NEO groups showed a statistically significant decline ($p < 0.05$) at the end of the experiment.

Table (3): The open field test, measuring the number of urination/ 3 minutes.

Groups N=8	Zero time (mean± S.E)	8 weeks (mean± S.E)
Control	0.34±0.72Aa	0.77±0.08Ab
Asp 500mg	1.37±0.64Bb	1.07±0.11Bb
Neo 500mg	1.09±0.79Bb	0.79±0.06Bb
LSD	0.67	

Different capital letters denote among groups statistical differences where small letters denote between periods statistical differences at ($p < 0.05$)

3.2. Swimming Rank Test

Table (4) showed the results after eight weeks of the trial, indicating that, compared to the control group, the swimming grade over 20 seconds significantly decreased in the ASP and NEO groups ($p < 0.05$).

Table (4): Swimming rank test (grade/20sec) of different treated groups.

Groups N=8	Zero time (mean± S.E)	8 weeks (mean± S.E)
Control	4.42±0.80Aa	4.31±0.50Bb
Asp 500mg	4.31±0.68Aa	2.89±0.31Aa
Neo 500mg	3.89±0.89Aa	1.19±0.39Ab
LSD	1.43	

Different capital letters indicate statistical differences among groups, while small letters denote statistical differences between time points ($p < 0.05$).

3.3. Y-Maze Test Alternate Arm/3min

Table (5) showed the y-maze results for the various treated groups, and at 8 weeks, all experimental groups showed a significant decrease ($p < 0.05$).

Table (5): Results of y-maze in the all-study groups.

Groups N=8	Zero time (mean± S.E)	8 weeks (mean± S.E)
Control	3.42±0.80Aa	3.01±0.60Bb
Asp 500mg	3.71±0.68Aa	2.67±0.41Aa
Neo 500mg	3.99±0.89Aa	1.99±0.78Ab
LSD	1.78	

Different capital letters indicate statistical differences among groups, while small letters denote statistical differences between time points ($p < 0.05$).

3.4. Hematological Parameters

Figure (2) showed the hematological parameters results for the various treated groups at 8 weeks (end of experiment) and all experimental groups showed a statistically significant variations ($p < 0.05$). RBC and WBC shown significant increase in ASP and NEO groups compare to control group but in Hct, Hb, and PLT shown significant decrease in ASP and NEO groups compared to control group ($p < 0.05$).

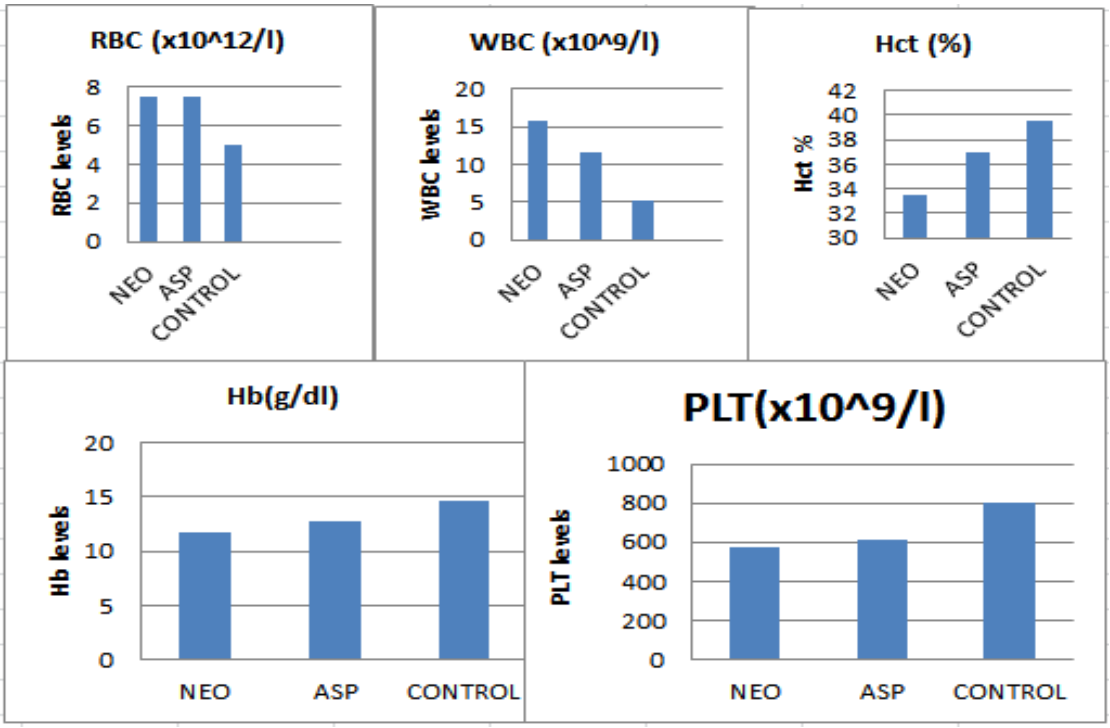

Figure (2): Effect of ASP and NEO on hematological parameters (x-axis represented the study groups (control, NEO, and ASP) and y-axis represented the hematological parameters levels (RBC: red blood cell; Hb: haemoglobin; Hct: haematocrit values; WBC: white blood cell; PLT: platelets).

Table (6) shows Effect of ASP and NEO on hematological parameters (RBC: red blood cell; Hb: haemoglobin; Hct: haematocrit values; WBC: white blood cell; PLT: platelets).

Table (6): Effect of ASP and NEO on hematological parameters.

Parameters	CONTROL mean±SD	ASP mean±SD	NEO mean±SD
RBC ($\times 10^{12}$/l)	5.03±1.09A	7.51±1.01B	7.54±0.99C
WBC ($\times 10^{9}$/l)	5.3±0.9A	11.6±0.67B	15.7±1.14C
Hb (g/dl)	14.7±1.56A	12.7±1.34B	11.8±1.23C
PLT ($\times 10^{9}$/l)	799±23A	608±21B	578±18C
Hct (%)	39.6±2.8A	36.9±2.5B	33.5±2.3C

Different capital letters denote among groups statistical differences ($p < 0.05$).

4. Discussion

With the increasing presence of sugar in a wide range of food products, global sugar consumption is on the rise, prompting the need for innovative approaches. The use of sweeteners to lower blood glucose levels, body mass, and calorie intake is an effective strategy for reducing the risk of chronic noncommunicable diseases [15]. Long-

term use of sugar-sweetened beverages has been increasingly linked to a number of negative health effects, including metabolic syndrome, type 2 diabetes, and obesity. Many medical professionals have suggested foods and drinks with noncaloric artificial sweeteners to lower the risk of various illnesses [16]. Aspartame (ASP), a non-nutritive sweetener, is commonly found in 'diet' and 'low-calorie' products, as well as in various foods, medications, and personal care items. Gut esterases and peptidases break down aspartame into three common chemicals: phenylalanine and aspartic acid, as well as trace amounts of methanol [17]. This study aimed to compare the effects of artificial sweeteners (ASP and NEO) on neurobehavioral and hematological parameters in rats. Recent studies have linked the consumption of artificial sweeteners to significant health hazards, according to recent studies. Although NEO is a relatively new sweetener on the global market, nothing is known about how it affects the commensal microbiota or intestinal epithelium [18]. The results of this research suggested that open field test measured the number of squares crossed by the four legs/3min. The administration of 500 mg/kb.bw of ASP and NEO significantly ($p<0.05$) reduced the number of squares crossed by four legs/3 minutes in both treatment groups of the study as compared to those in zero time and by the end of the eight-week experiment, the results were statistically significant compare to control group (p-value< 0.05). The paper investigates the neurobehavioral effects of aspartame or neotame (or both) on rats in the open field test. This includes any measured changes in behavior (e.g., locomotion, anxiety) as a result of exposure to these artificial sweeteners. Souto *et al*., 2023 investigated the deleterious effects at the central nervous system of studies rats caused by administration of 75 mg/kg.bw of ASP [19]. Ashok & Sheeladevi, 2015 founds that ASP alters the function in the brain by elevating the reactive oxygen species (ROS) and activates the apoptotic pathway in the brain neurons and these because of changes may be due to the methanol or its metabolite of ASP and the researchers examined the neurobehavioral effects of ASP in rats, specifically focusing on anxiety-like behavior and locomotor activity in the open field test. It also examines the activation of neurodegenerative apoptosis in the rat brain following long-term aspartame consumption [20]. The results of this study presented that there was no statistically significant difference ($p<0.05$) in the number of fecal boluses every 3 minutes among the study groups. Research on aspartame's effects on gut function has highlighted several impacts on fecal characteristics and gastrointestinal function in rodent models. Despite being considered safe due to minimal metabolism by human digestive processes, artificial sweeteners can be actively metabolized by gut microbiota, potentially producing harmful metabolites [21]. Aspartame exposure has been shown to influence bacterial pathogenicity in gut microbiota models. Shil *et al*., 2024 demonstrated that aspartame exposure increased biofilm formation in model gut bacteria (E. coli and E. faecalis), enhancing their ability to adhere to, invade, and damage intestinal epithelial cells and this suggests that aspartame may potentially disrupt normal host-microbiota interactions [22]. The physiological quantities of neotame that the intestinal epithelium and microbiota would be exposed to in a typical diet are difficult to determine because artificial sweeteners are found in a wide variety of food and drink products used by the general public [23].

Both aspartame (ASP) and neotame (NEO) decreased hemoglobin levels, hematocrit values, and red blood cell count as compared to controls. This raises the possibility that these artificial sweeteners could cause anemia. Other investigations have documented similar hematological changes in which ASP induced oxidative stress and interfered with erythropoiesis, potentially through a decrease in antioxidant enzyme levels and an increase in lipid peroxidation [24]. Anemia may result from iron metabolism disruption, oxidative damage to red cell membranes, or bone marrow suppression [25]. In the groups treated with ASP and NEO, the WBC count was higher, suggesting a potential immunological or inflammatory reaction. A rise in WBC could indicate the body's response to systemic stress or tissue damage, possibly as a result of sweeteners being metabolized into formaldehyde or other reactive intermediates [26]. Both treatment groups experienced a significant decrease in platelet count. Thrombocytopenia may indicate altered thrombopoiesis or bone marrow toxicity, and it may also raise the risk of bleeding. This is consistent with studies that bone marrow-derived cells may be toxically affected by artificial sweeteners [27].

The results of this study align with recent literature that raises concerns about the potential neurobehavioral effects of artificial sweeteners. For instance, a study by Pang et al., 2021 found that long-term consumption of aspartame was associated with behavioral alterations in rodents, suggesting neurotoxic potential [23]. Similarly, research by Smith and López-Meza et al., 2022 indicated that neotame may have comparable effects, impacting cognitive functions and behavior in animal models [28].

The observed decrease in locomotor activity and swimming performance among the treated groups could be attributed to the neurotoxic effects of these sweeteners [29]. Previous research has indicated that aspartame can result in alterations to neurotransmitter levels, potentially affecting motor functions and behavior. Furthermore, the hematological changes observed in this study are consistent with findings reported by Wilk et al., 2022 [30] who noted that aspartame consumption led to significant changes in blood parameters, suggesting possible adverse effects on overall health and body weight. The implications of these findings are significant, considering the widespread use of artificial sweeteners in food products.

5. Conclusions

The present study demonstrates that both aspartame and neotame have detrimental effects on neurobehavioral and hematological parameters in male rats. Potential systemic toxicity involving oxidative stress and hematopoietic disruption is suggested by the hematological changes seen in rats after ASP and NEO treatment. These results call into question safety, especially when chronic exposure occurs, and emphasize the necessity of cautious intake and more human toxicological testing. The significant changes observed in locomotor activity, swimming performance, and blood parameters suggest that these artificial sweeteners pose health risks that warrant further investigation. Future research should focus on elucidating the mechanisms behind these effects and assessing the long-term implications for human health, especially given the rising prevalence of artificial sweeteners in our diets.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

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