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Effect of Chronic Neotame Exposure on Visfatin Level, Oxidative Stress Biomarkers, and Small Intestine Tissue in Male Rats

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Abstract

Background: Neotame (NEO) is a potent artificial sweetener widely used as a low-calorie sugar substitute. Although considered safe at recommended levels, prolonged exposure may contribute to physiological and biochemical disturbances through oxidative stress and free radical production, potentially resulting in dose- and duration-dependent cellular and functional damage.

Objectives: This study aimed to investigate the effects of chronic neotame administration on oxidative stress biomarkers (GSH and MDA), serum visfatin levels, and histopathological alterations in the small intestine.

Materials and Methods: A total of 40 healthy adult male albino rats (180–230 g, 60–75 days old) were housed in the animal house of the College of Science, University of Babylon. The animals were acclimatized for two weeks under controlled laboratory conditions (20–25°C, 12 h light/dark cycle) with free access to food and water, The experiment began on the first of December, 2025, and ended on the first of March, 2026.

Results: Statistical analysis revealed no significant differences in serum visfatin levels among the experimental groups. In contrast, oxidative stress biomarkers showed significant alterations following chronic neotame administration, with a significant decrease ($P \leq 0.05$) in glutathione (GSH) levels and a significant increase ($P \leq 0.01$) in malondialdehyde (MDA) levels in a dose-dependent manner, indicating enhanced oxidative stress and lipid peroxidation. Histopathological examination of the small intestine demonstrated dose-related tissue alterations, including goblet cell proliferation, villous atrophy and sloughing, inflammatory cell infiltration, vascular congestion, crypt hyperplasia, villous fusion, and necrosis of intestinal glands, with more severe lesions observed at higher neotame doses.

Conclusion: The study reveals that continuous exposure to neotame leads to dose-dependent physiological, Histopathological, and biochemical alterations.

Keyword: Neotame; Artificial sweeteners; Visfatin; Oxidative stress; Small intestine histopathology and Albino rats

1. Introduction

Artificial sweeteners are widely used as sugar substitutes in foods and beverages because they provide sweetness with little or no caloric value. They are commonly incorporated into low-calorie products to assist in body weight management and glycemic control. These compounds exert their effects by activating sweet taste receptors and may also influence metabolic processes within the body [1].

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Neotame is a high-intensity artificial sweetener derived from aspartame and is several thousand times sweeter than sucrose, owing to its high sweetening potency, chemical stability, and negligible caloric contribution, it has been extensively utilized in a variety of food products since its approval for commercial use, although considered safe within established intake limits, concerns remain regarding the potential biological effects associated with long-term consumption, necessitating further investigation [2].

Oxidative stress results from an imbalance between the production of reactive oxygen species and the body's antioxidant defense systems, malondialdehyde (MDA), a major end-product of lipid peroxidation, is widely used as a biomarker of oxidative damage because elevated levels reflect increased oxidation of cellular membrane lipids and disruption of redox homeostasis[3].

Glutathione (GSH) is one of the most important intracellular antioxidants, playing a central role in protecting cells against oxidative injury, it participates in detoxification processes, supports antioxidant enzyme activity, and contributes to the maintenance of cellular redox balance, thereby preserving cellular integrity and normal physiological function [4].

Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT), is an adipokine predominantly produced by visceral adipose tissue and immune cells, it is involved in glucose metabolism, inflammatory regulation, and cellular energy homeostasis, altered visfatin levels have been associated with several metabolic and cardiovascular disorders, highlighting its potential value as a biomarker of metabolic dysfunction and inflammation [5].

Research Objectives

- The goal of this study is to examine the impact of the artificial sweetener neotame on physiological, biochemical, and histological parameters in adult wistar male rats at varying concentrations, in particular, the investigation concentrates on:
- Assessment of oxidative stress indicators (MDA and GSH) after neotame exposure.
- Assess the influence of neotame on metabolic activity.
- Examining histological alterations in small intestine tissues induced by neotame.

2. Materials and methods

2.1. Experimental Animals

Forty healthy adult male albino rats (180–230 g, 60–75 days old) were acclimatized for two weeks and housed under standard laboratory conditions (20–25°C, 12 h light/dark cycle) with ad libitum access to food and water.

2.2. Design of Experiment:

In this study, 40 male animals Wister rats were divided randomly into four groups each group included 10 animals:

- Group (CONT) : Administration of normal distal water for 12 weeks orally.
- Group 1 : Administration of Neotame (250 mg/kg/day) dissolve in distal water for 12 weeks orally.
- Group2 : Administration of Neotame (500 mg/kg/day) dissolve in distal water for 12 weeks orally.
- Group 3 : Administration of Neotame (750 mg/kg/day) dissolve in distal water for 12 weeks orally.

2.3. Sample Collection and Biochemical Analysis

Twenty-four hours after the final treatment, rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) administered intramuscularly, blood samples were collected by cardiac puncture, centrifuged at 3000 rpm for 15 min, and the obtained serum was stored at −4°C for subsequent biochemical analyses.

2.4. Histopathological Examination

At the end of the experiment, the rats were perfused with ketamine (80 mg/kg) and xylazine (12 mg/kg), small intestine tissues were harvested and preserved in 10% neutral buffered formalin. Tissue samples were treated for histological evaluation by the method of [6], which involved dehydration in graded ethanol, clearing in xylene, paraffin embedding and sectioning at 5 µm thickness, sections were stained with hematoxylin and eosin (H&E) according to method reported by [7], mounted with DPX and inspected under light microscopy for histological examination.

2.5. Statistical Analyses

Statistical analyses were conducted using SPSS version 26. One-way ANOVA followed by the LSD test was applied to compare group means. A probability level of $P \leq 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Effect Neotame on Visfatin Level

The present study demonstrated that no significant differences ($p > 0.05$) in visfatin levels were observed among the neotame-treated groups compared with the control group, although a slight dose-dependent increase was noted, the changes remained statistically non-significant, indicating that chronic neotame administration had no measurable effect on circulating visfatin levels, as shown in figure (1)

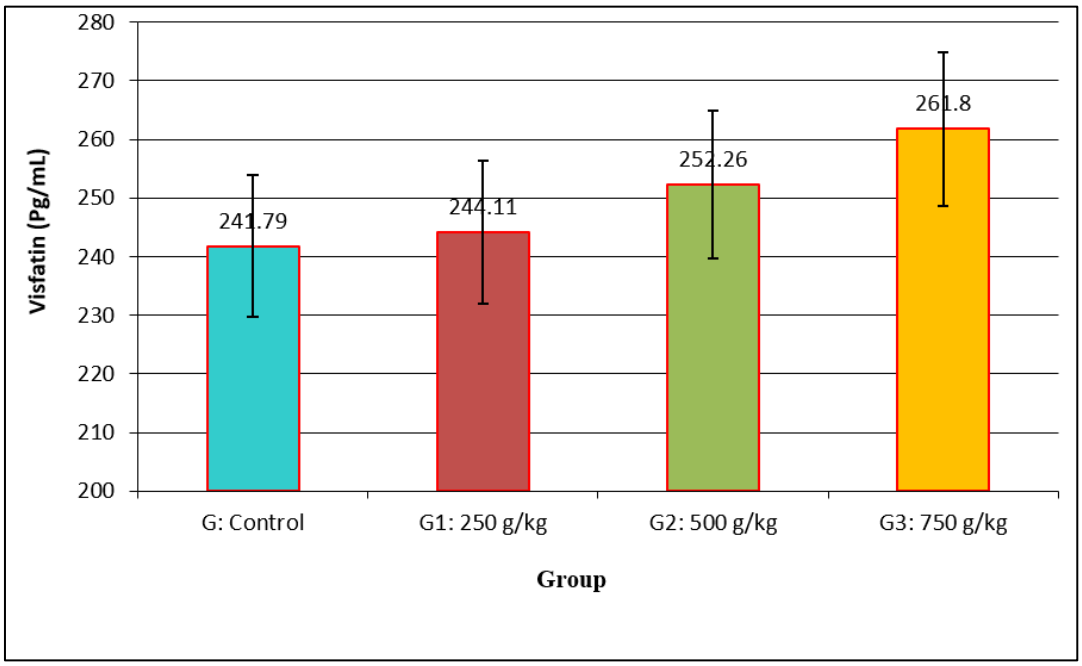

Figure 1 Effect of Different Doses of Neotame on Visfatin level

This finding is consistent with evidence suggesting that visfatin regulation is primarily linked to metabolic disturbances such as obesity, insulin resistance, and inflammation rather than exposure to individual dietary components[8,9,10]. Nevertheless, previous studies have reported that artificial sweeteners may induce metabolic alterations through changes in gut microbiota composition, glucose metabolism, and inflammatory pathways, which could potentially influence adipokine secretion, including visfatin [11,12,13].

3.2. Effect Neotame on Oxidative Stress

The present study demonstrated that chronic exposure to high doses of neotame significantly affected oxidative stress and antioxidant biomarkers, as reflected by decreased glutathione (GSH) levels and increased malondialdehyde (MDA) levels. GSH showed a dose-dependent reduction in treated groups, with a significant decrease observed at the highest dose compared with the control group ($P \leq 0.05$), indicating a suppression of the antioxidant defense system following prolonged neotame exposur, as shown in figure (2).

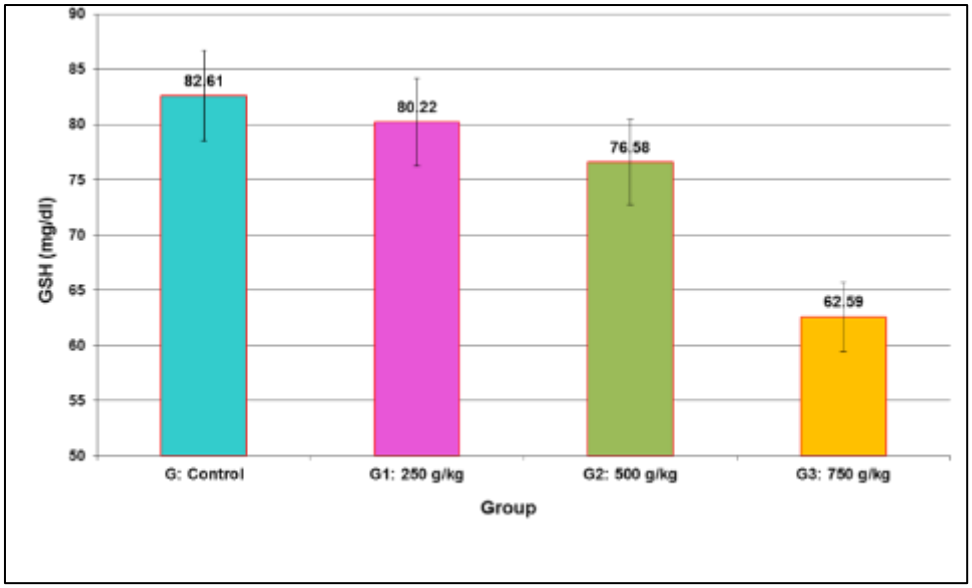

Figure 2 Effect of Different Doses of Neotame on GSH Concentration

In contrast, MDA levels increased progressively with neotame administration, with a significant elevation observed in the high-dose group ($P \leq 0.01$), suggesting enhanced lipid peroxidation and oxidative damage, as shown in figure (3).

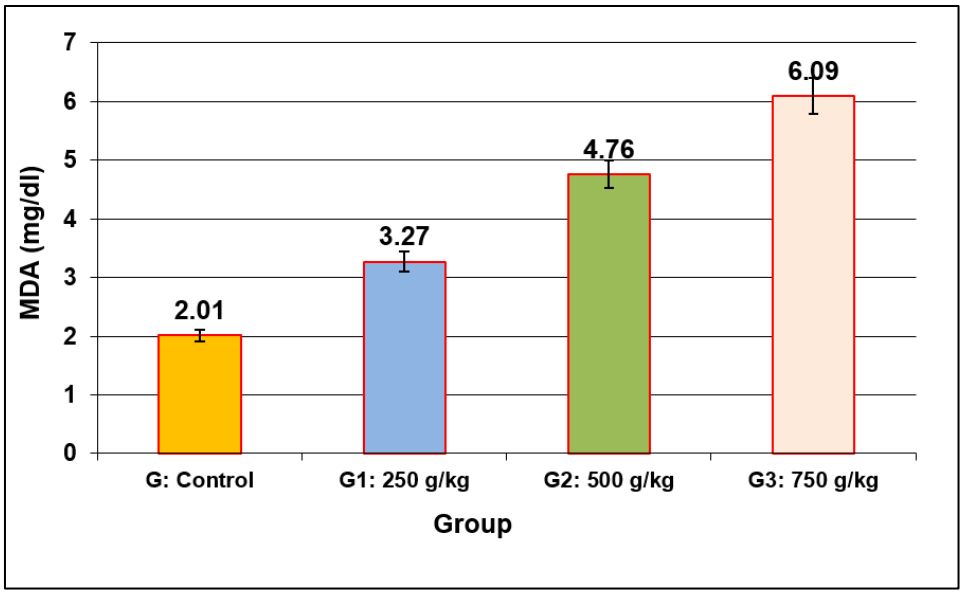

Figure 3 Effect of Different Doses of Neotame on MDA Concentration

These findings are consistent with previous studies reporting that non-nutritive sweeteners may alter gut microbiota composition and host metabolic pathways, leading to increased oxidative stress and reduced antioxidant capacity [11,13]. However, conflicting evidence exists, as some studies have reported minimal or no significant oxidative effects under normal physiological conditions, with outcomes strongly influenced by dosage, duration of exposure, and experimental model [14,15,16].

3.3. Effect Neotame on Small Intestine Tissues

The neotame-treated groups exhibited histopathological and inflammatory alterations in the small intestinal tissue, characterized by dose-dependent inflammatory responses that progressively increased with higher concentrations, these changes also included villus atrophy, crypt hyperplasia, and necrosis of intestinal glands when compared with normal intestinal architecture, these findings are consistent with[11], who reported that non-nutritive sweeteners may disrupt gut microbiota homeostasis, leading to activation of intestinal inflammatory responses, as shown in figure (4),

(5), (6), (7),(8). In contrast, the control group demonstrated normal small intestinal histoarchitecture with no observable signs of inflammation or pathological lesions, confirming that the observed alterations in the treated groups are attributable to neotame exposure rather than external factors, this observation aligns with[17], who emphasized that intestinal barrier integrity is closely associated with a stable microbiota and the absence of disruptive dietary influences.

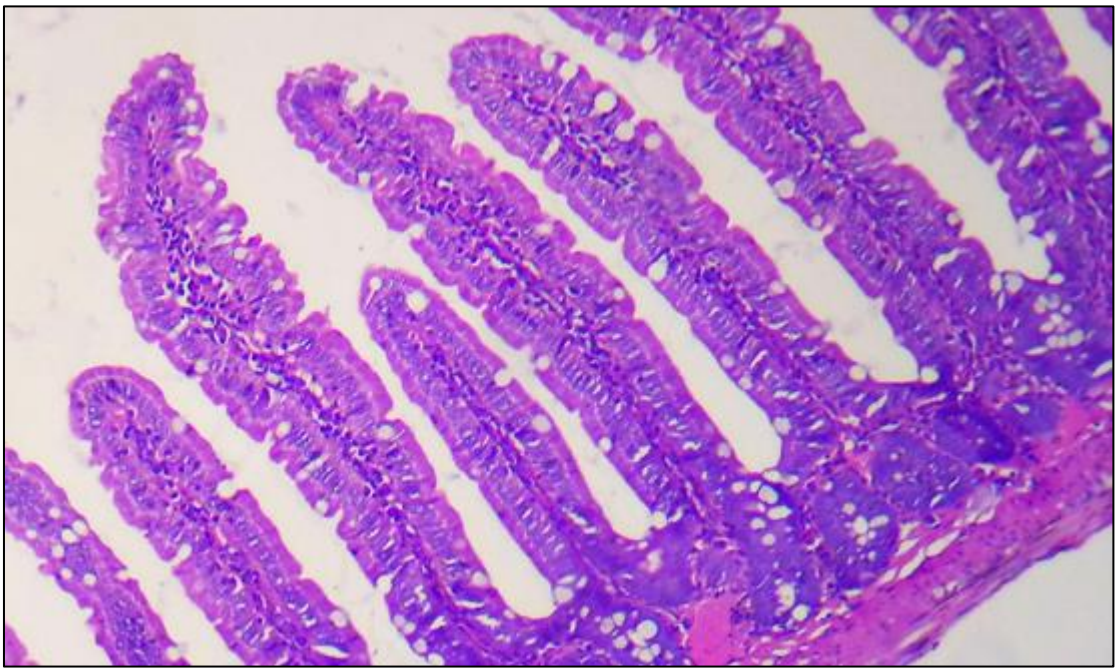

Figure 4 Histological section of small intestine for control group showing normal histological structures. (H & E), 20x

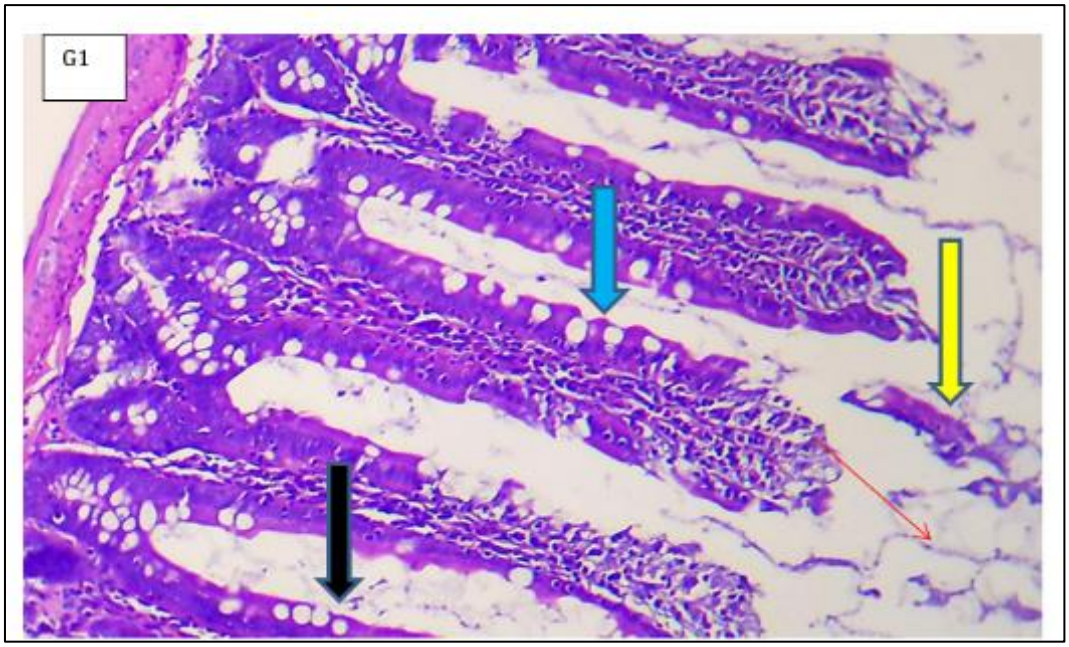

Figure 5 Histological section of rat of small intestine for G1 showing increase in goblet cells(black arrow) and increase in mucin secretion (red arrow), sloughing in some intestinal villi (yellow arrow) with in flammtory cells infiltration (blue arrow). (H & E), 20x

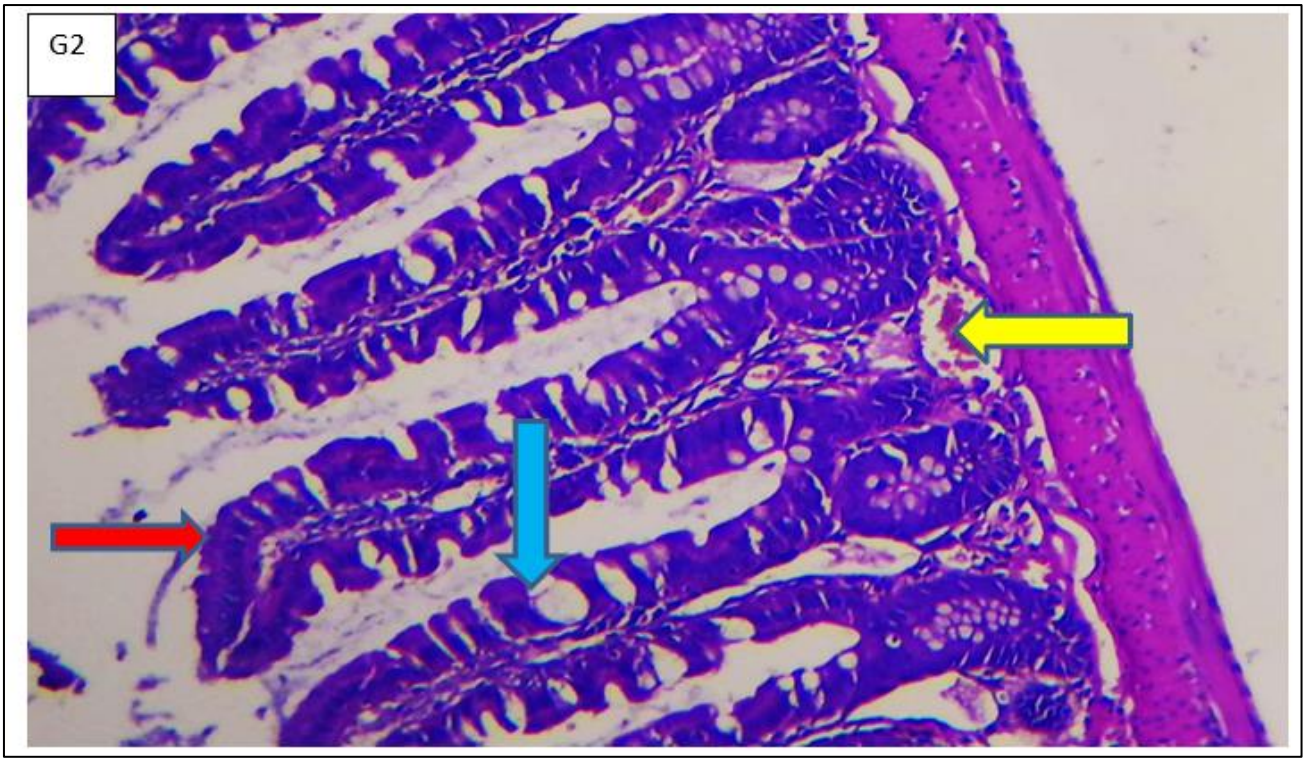

Figure 6 Histological section of rat for G2 showing increase in goblet cells proliferating (blue arrow), atrophy of villus (red a rrow), with congesting of blood vessels (yellow arrow). (H & E stain), 20x

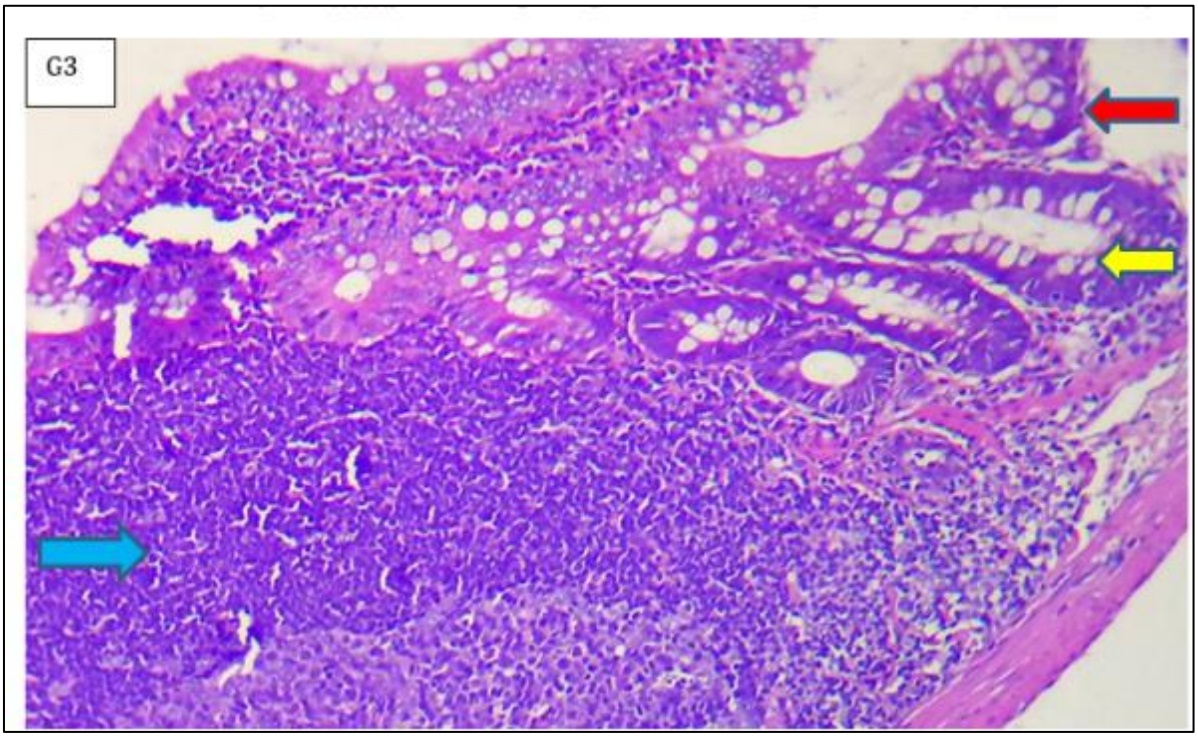

Figure 7 Histological section of rat of small intestine of G3 showing sever inflammatory cells infiltrating in submucosa (blue arrow), atrophy of villi (red arrow) with crypts hyperplasia (yellow arrow). (H & E), 20x

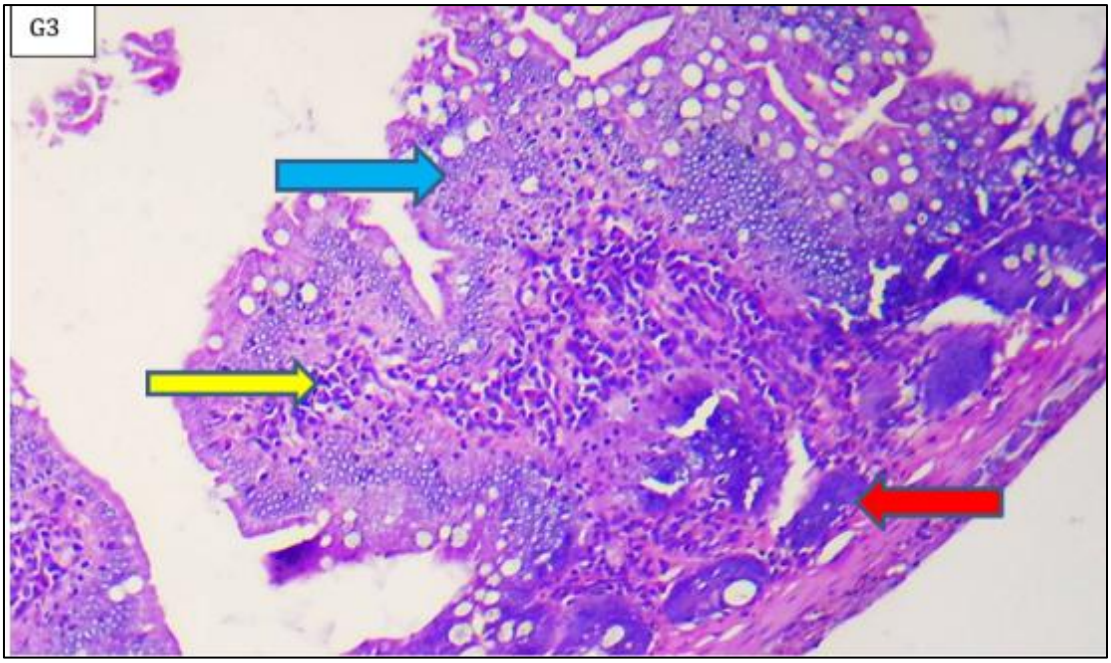

Figure 8 Histological section of rat of small intestine for G3 showing fusion and atrophy of intestinal villi(blue arrow) with necrosis of intestinal glands (red arrow), in flammation Cell infiltrating also seen (yellow arrow). (H & E),20x

Furthermore, previous studies have indicated that dysbiosis induced by artificial sweeteners may increase intestinal permeability and trigger local inflammatory pathways, which could explain the severity of histological alterations observed in the treated groups compared with the control [18]. In addition, recent evidence suggests that neotame may compromise intestinal epithelial integrity and induce tissue damage through both microbiota-dependent and direct effects on epithelial cells [19, 20].

4. Conclusion

Chronic exposure to neotame caused significant oxidative stress in male Wistar rats as evidenced by significant reduction in reduced glutathione (GSH) concentrations and increase in malondialdehyde (MDA) levels indicating increased lipid peroxidation and disruption of the antioxidant defense system. Furthermore, histological changes were found in the small intestinal tissues which imply possible structural and functional harm after long term neotame use. These data together indicate that neotame may have deleterious effects on redox homeostasis and intestinal integrity in a dose-dependent manner.

Recommendations

- Further research are necessary to investigate the long-term effects of neotame on different bodily systems, such as digestive systems.
- Histopathological study of the effects of neotame on different body organs.
- Evaluation of the long-term effects of neotame on female rats.
- Future studies should examine additional biomarkers that are linked to cellular inflammation and oxidative stress.
- Excessive and extended usage of artificial sweeteners should be addressed with caution due to their possible detrimental health effects.

Compliance with ethical standards

Disclosure of conflict of interest

This study without any conflict of interest

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