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Impact of Some Natural and Artificial Sweeteners Consumption on Different Hormonal Levels and Inflammatory Cytokines in Male Rats: In Vivo and In Silico Studies

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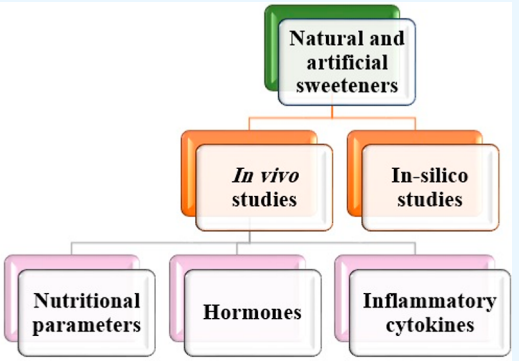
ABSTRACT: Substituting sugar with noncaloric sweeteners prevents overweight and diabetes development. They come in two types: artificial, like aspartame and sucralose, and natural, such as sorbitol. This research aimed to assess the effects of sucrose and these sweeteners on nutritional parameters, hematological parameters, hormones, and anti- and pro-inflammatory cytokines in male rats. Thirty rats had been separated into five groups. The results showed the highest significant increase in body weight gain, total food intake, and feed efficiency noticed in the aspartame group followed by sucralose, sucrose, and sorbitol, respectively. In contrast to RBCs and platelets, all sweeteners significantly reduced the hemoglobin level, Hct %, and WBC count. The aspartame group showed the highest decline in glycoproteins, steroids, and T3, and T4 hormones and a dramatic elevation in thyroid stimulating hormone, eicosanoid, and amine hormones compared with the control group. A vigorous elevation in anti- and proinflammatory cytokine levels was observed in the aspartame group, followed by sucralose, sucrose, and sorbitol groups. Aspartame has the highest docking scores when studying the interactions of sweeteners and a target protein associated with hormones or cytokines using in silico molecular docking, with the best absorption, distribution, metabolism, elimination, and toxicity properties compared to the remaining sweeteners.



1. INTRODUCTION

The consumption of sugars is increasing worldwide due to their prevalence in a wide range of dietary items, and new strategies have been spurred. An effective approach involves using sweeteners to decrease calorie consumption, body mass, and blood glucose levels, hence mitigating the risk of chronic noncommunicable disorders.[1] Sweeteners are substances added to food or drinks to sweeten them and emulate sugar taste. They may be categorized as natural, like sucrose and sorbitol, or artificial, such as aspartame and sucralose. They can also be categorized as either nutritive, having an energy intake similar to sugar, or non-nutritive, as they do not provide energy to the body.[2]

Sweeteners have diverse effects on cellular pathways, hormone levels, and glycemia, but the literature presents contradictory information. Some studies suggest sucralose can hinder the body's inflammatory response and increase vulnerability to external pathogens.[3,4] On the other hand, sweeteners containing nutrients like sucrose can boost the cellular inflammatory response and help protect the body against infectious agents.[5] Sucralose can enhance the progression of tumors and disrupt the balance of gut bacteria, hinder the deactivation of digesting protease, harm the integrity of the barrier to the gut, and worsen inflammation. Aspartame consumption should be regulated due to its negative impact on oxidative stress levels, antioxidant production, and brain health. Studies have shown that it increases oxidative stress markers while decreasing antioxidant defenses, leading to inflammatory markers and potential health risks like obesity, cardiovascular disease, and cancer.[6] Depending on the study done by Mongkhon et al.,[7] sorbitol may function as an oxidant and an antioxidant by inhibiting the synthesis of catabolic, inflammatory, and oxidative stress-related mediators caused by IL-1β and the indicators of apoptosis generated by H_2O_2.

Studies have shown mixed findings regarding the effect of sweeteners on hormonal levels. Sucralose raises body weight, decreases food consumption, and lowers glucose insulinotropic

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peptide (GIP) release and glycemia without changing insulin concentration.[8] In contrast, unlike water controls, non-nutritive sweeteners in beverages (alone or in blends) do not significantly impact glucose, insulin, GLP-1, GIP, PYY, ghrelin, and glucagon responses. Consuming foods high in caloric sugars, such as glucose and sucrose, can increase the body's glucose, insulin, GLP-1, and GIP levels, while ghrelin and glucagon responses remain the same. There are no significant differences in the postprandial glycemic and endocrine effects between the coupling and delayed coupling treatments' non-nutritive sweeteners and controls.[9] Aspartame can harm the hypothalamic-pituitary-gonadal axis in female mice, which can reduce the function of the ovaries and reproductive hormone feedback mechanisms.[10] In line with the previous study, Anbara et al.[11] and Abbas et al.[12] approved that long-term aspartame consumption resulted in reproductive damage in male mice through oxidative stress induction. Revealed that aspartame increased insulin, testosterone, T3, and T4 hormone levels. They suggest avoiding artificial sweeteners due to their potentially harmful effects on hormones, lipid and protein profiles, and other physiological parameters. It was established that sorbitol and thyroid hormone status were correlated, with sorbitol elevating thyroid stimulating hormone (TSH), T3, ATP, and glucose levels and decreasing triglyceride and cholesterol levels.[13]

To our knowledge, no studies were found for the complete profiles of protein, peptide, glycoprotein, eicosanoid, steroid, and amine hormones in addition to anti- and pro-inflammatory cytokines in male rats due to sweeteners consumption. Therefore, it is necessary to clarify the contradictions presented in the published literature in order to have a complementary study concerning the relations among different types of hormones, inflammation, and nutritional parameters due to the consumption of sweeteners. Consequently, the present study aims to evaluate the effects of aspartame and sucralose as artificial sweeteners and sucrose and sorbitol as a natural on various experimental male rats' parameters such as water intake, nutrition, hematology, hormones, and anti- and pro-inflammatory profile. In silico molecular docking helps predict the interaction between a ligand or sweetener and a target protein associated with hormones or cytokines. Absorption, distribution, metabolism, elimination, and toxicity (ADMET) evaluation models are used to assess sweeteners' safety. In silico studies, which represent a novelty point in the current research, help understand the biological actions of sweeteners against hormones and cytokines.

2. MATERIALS AND METHODS

2.1. Materials. Analytical chemicals aspartame, sucrose, sucralose, and sorbitol were provided by Sigma-Aldrich (St. Louis, MO, USA). The rats' meals were supplemented with unsaturated fat, sugar, and maize starch from a local market. The ELISA kits were purchased from Fine Test (Wuhan Fine Biotech Co., Ltd.) in Wuhan, China, and Sunlong Biotech Co. in Yuhang, China.

2.2. Methods. *2.2.1. Experimental Animals.* Thirty male Sprague−Dawley rats, with an average weight of 180 ± 20 g and an age of 2 months, were obtained from the Animal House Colony of the National Research Centre in Cairo, Egypt. Before the experiment, the animals underwent a week-long period, during which they were provided with a regular laboratory diet consisting of food and water. This was done to facilitate their adjustment to the environment and ensure that their growth and behavior were suitable for the experiment. The animals were distributed and housed in cages with solid bottoms in a chemical-free room, temperature-controlled at 23 °C, had regulated relative humidity between 40 and 60%, and were artificially lighted with a 12 h dark/light phase. The Egyptian National Research Center's animal research protocol was authorized by the Ethical Committee of Medical Research (Approval no. 74910112022). The treatment of all animals adhered to the UK's Animals (Scientific Procedures) Act, 1986, its accompanying recommendations, and EU Directive 2010/63/EU for animal research (Publication no. 85−23, updated 1985), ensuring their humane handling and usage by national and international regulations.

2.2.2. Diet Composition. Casein (150 g/kg diet), unsaturated fat (100 g/kg diet), sucrose (220 g/kg diet), starch from maize (440 g/kg diet), cellulose (40 g/kg diet), salt mixture (40 g/kg diet), and vitamin mixture (10 g/1 kg diet) were the components of the synthetic base diet, according to refs 14 and 15. The AIN-93 M diet was a basis for creating salt and vitamin combinations.[16]

2.2.3. Experimental Design. Thirty rats will be arranged into five groups (n = 6 rats/each) as described below:

- Normal control: rats were fed the basic balanced diet daily, and normal saline was administered orally.
- Aspartame group: rats were fed on the synthetic base diet and given aspartame dissolved in distilled water orally for 12 weeks (40 mg/kg bw/day).[17]
- Sucralose group: rats were fed on the synthetic base diet and sucralose dissolved in distilled water, which were given orally for 12 weeks (5 mg/kg bw/day).[18]
- Sucrose group: rats were fed on the synthetic base diet and sucrose dissolved in distilled water, which were given orally for 12 weeks (41.66 mg/mL).[8]
- Sorbitol group: rats were fed on the synthetic base diet and sorbitol dissolved in distilled water, which were given orally for 12 weeks (50 mg/kg bw/day).[19]

All of the doses of natural and artificial sweeteners were given as an acceptable daily intake (ADI) according to European Food Safety Authority (EFSA).[20]

2.2.4. Determination of Body Weight, Food Consumption, and Water Consumption. The basal synthetic diet was given to all of the protocol groups. Each rat was fed about 20 g/day. Water was given daily for 24 h until the end of the experiment. The body weight was measured weekly using a digital weighing balance in order to evaluate the weekly weight changes. The water consumption of rats was quantified daily by subtracting the quantity remaining (mL) from the quantity supplied (mL) for 12 weeks. Also, the fluid bottles were examined for any indication of leakage or blockage. On the other hand, the quantification and computation of food consumption were conducted utilizing metabolic cages and digital weighing balances.

2.2.5. Blood Sample Collection. Following the three-month research period, the animals fasted for 12 h, were administered pentobarbital anesthesia at a dosage of 80 mg/kg, had blood drawn directly from the heart using a syringe containing heparin, and were then euthanized by cervical dislocation. The serum and plasma were isolated from the blood samples using centrifugation (Sigma Labor Centrifuge GMBH, Germany, model 2-153360 Osterode/Hz) at 4000 rpm per minute for 15 min. The separated samples were then stored at a temperature of −20 °C.

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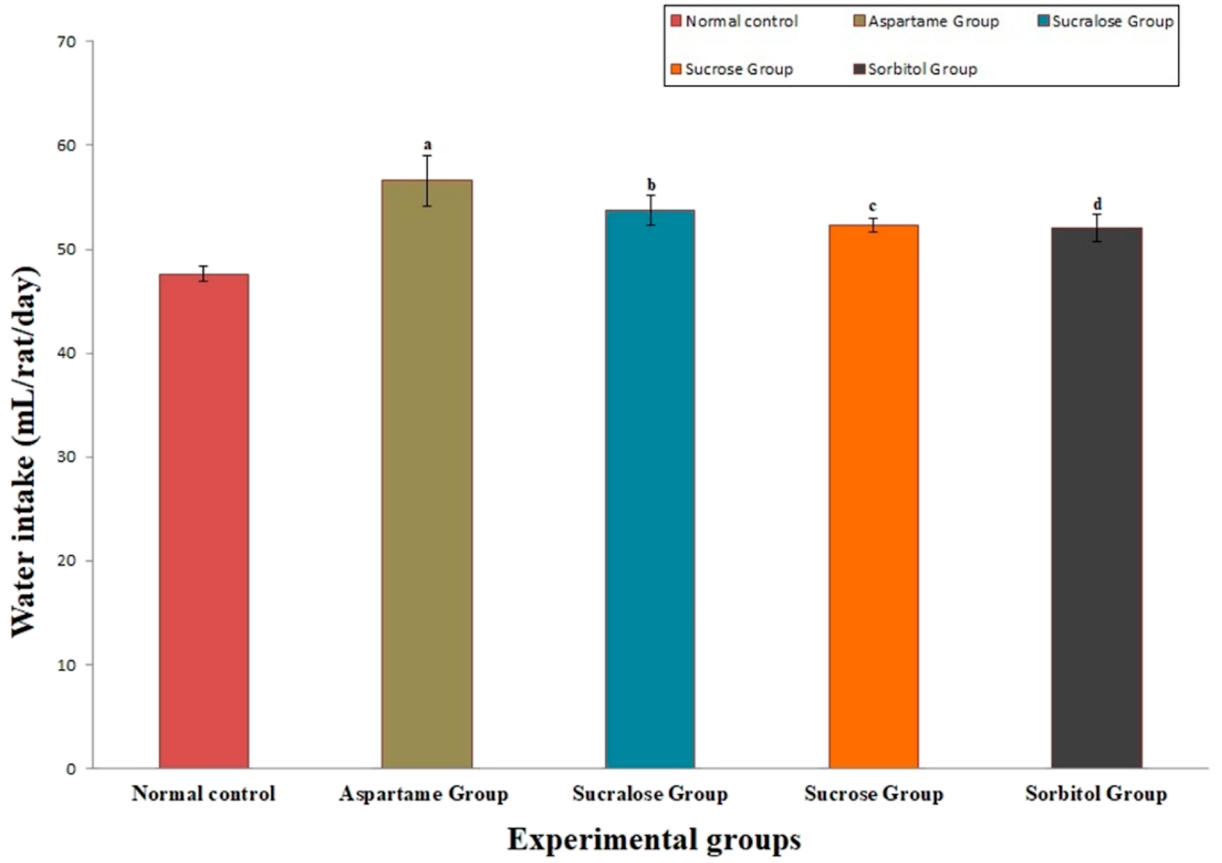

Figure 1. Consumption of water with natural and artificial sweeteners.

2.2.6. Hematological Methods. Hematological examination was conducted using whole blood samples supplemented with EDTA. The hematological parameters, namely, red blood cells (RBC), white blood cells (WBC), platelets, hemoglobin (Hb), and hematocrit (Hct %), were measured in whole blood using conventional procedures on an automated hematology analyzer.

2.2.7. Biochemical Parameters. 2.2.7.1. Protein and Peptide Hormones. Insulin, Glucagon, Leptin, Amylin, GIP, and GLP-1 were evaluated using ELISA kits following the manufacturer's instructions.

2.2.7.2. Glycoprotein Hormones and Eicosanoid Hormone. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), TSH, parathyroid hormone (PTH), and prostaglandin E2 were evaluated using ELISA kits following the manufacturer's instructions.

2.2.7.3. Steroid and Amine Hormones. Testosterone, progesterone, estradiol, T3, T4, epinephrine, and norepinephrine were evaluated using ELISA kits following the manufacturer's instructions.

2.2.7.4. Anti- and Pro-inflammatory Cytokines. Tumor necrosis factor (TNF-α), IFN-γ, Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Interleukin-1β (IL-1β) were evaluated using ELISA kits following the manufacturer's instructions.

2.2.8. Molecular Docking. The following crystal structures of enzymes and receptors were obtained from the PDB: protein data bank (https://www.rcsb.org/, accessed on January 31, 2022, October 29−30, 2023, and November 5, 16−18, 2023): glutamine fructose-6-phosphate amidotransferase (PDB ID: 2ZJ3), leptin receptor (PDB ID: 3V6O), human thyroid hormone receptors β and α (PDB IDs: 3GWS and 4LNX), thyrotropin (TSH) receptor (PDB ID: 1XZX), human glucagon receptor (PDB ID: 5EE7), dipeptidyl peptidase IV (PDB ID: 5KBY), human amylin (PDB ID: 2L86), beta subunit of LH (PDB ID: 6P57), superoxide dismutase (PDB ID: 3HW7), glutathione peroxidase 4 (PDB ID: 2OBI), glutathione reductase (PDB ID: 1XAN), catalase (PDB ID: 1QQW), human FSH (PDB ID: 1FL7), human calcium-sensing receptor extracellular domain (PDB IDs: 5K5S), β1 and β2 adrenergic receptors (PDB IDs: 2VT4 and 2R4R), human α-adrenergic receptor (PDB ID: 6KUW), glucocorticoid receptor (PDB ID: 6NWK), human placental aromatase cytochrome P450 (PDB ID: 5JKV), human 3β-hydroxysteroid dehydrogenase (PDB ID: P14060), cytochrome P450 17A1 (PDB ID: 3RUK), 15-hydroxyprostaglandin dehydrogenase (PDB ID: 8CVN), and 15-keto prostaglandin-13-reductase (PDB ID: 2ZB4). The receptors were prepared by removing cocrystallized ligands and ions and protonating the remaining molecule using the Pymol program (Ver. 2.5.1). The ligands' 3D structures downloaded from the PubChem database (http://pubchem.ncbi.nlm.nih.gov, accessed on January 13, 2022) were optimized using Avogadro Software (Version 1.2.0) and MMFF94 force field.[21] Blind docking was performed using CB-Dock (http://clab.labshare.cn/cb-dock/php/, accessed on January 31, 2022, October 29−30, November 4−6, and 16−19, 2023), which predicted protein cavities and computed their top N centers and diameters.[22] After the pdbqt files were sent to AutoDock Vina for docking, N compute cycles were needed to achieve the final findings. Liu et al.[22] showed that top-ranking poses had success rates of rmsds less than 2 Å from their location in the X-ray crystal

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Table 1. Effect of Natural and Artificial Sweeteners on Nutritional Parameters[a]

groups	initial body weight (g)	final body weight (g)	body gain (g)	total food intake (g)	feed efficiency
normal control	184.5 ± 3.83	306.7 ± 5.16	122.2 ± 1.33	10681.7 ± 2.58	0.011 ± 0.516
aspartame group	183.7 ± 2.66[a]	390.3 ± 5.28[a]	206.6 ± 2.62[a]	10745 ± 18.71[a]	0.019 ± 0.14[a]
sucralose group	181.7 ± 4.08[a]	386.3 ± 4.27[b]	204.6 ± 0.16[b]	10690 ± 14.14[a]	0.019 ± 0.011[b]
sucrose group	179.7 ± 3.14[a]	376.3 ± 4.59[c]	196.6 ± 1.45[c]	10683.3 ± 18.62[a]	0.018 ± 0.078[c]
sorbitol group	179.3 ± 3.39[a]	371.5 ± 5.21[d]	192.2 ± 1.82[d]	10681.7 ± 11.69[a]	0.018 ± 0.156[d]

[a]The values were shown as mean ± SD (n = 6), where different letters indicate a significant difference at $P < 0.05$, and the same letters in each column indicate a nonsignificant difference between categories. Feed efficiency= (body gain/total food intake).

Table 2. Effect of Natural and Artificial Sweeteners on the Haematological Parameters[a]

	groups				
parameters	normal control	aspartame group	sucralose group	sucrose group	sorbitol group
RBC (x 1012/L)	5.13 ± 0.29	6.1 ± 0.4[a]	5.77 ± 0.34[b]	5.43 ± 0.15[c]	5.07 ± 0.53[d]
Hb (g/L)	116.73 ± 0.26	97.40 ± 0.23[a]	97.53 ± 0.69[b]	98.23 ± 0.16[c]	98.5 ± 0.21[d]
Hct (%)	39.3 ± 0.49	35.55 ± 0.21[a]	36.1 ± 0.64[b]	36.35 ± 0.18[c]	36.73 ± 0.45[d]
WBC (x 109/L)	4.7 ± 0.25	3.43 ± 0.16[a]	3.57 ± 0.23[b]	3.68 ± 0.42[c]	3.90 ± 0.6[d]
platelets (x 109/L)	575.15 ± 2.93	668.08 ± 2[a]	661.78 ± 1.52[b]	656.4 ± 5.83[c]	651.75 ± 0.55[d]

[a]The values were shown as mean ± SD (n = 6), where different letters indicate a significant difference at $P < 0.05$, and the same letters in each row indicate a nonsignificant difference between categories.

structure. Discovery Studio software version 21.1.0.20298 is used to analyze the best-docked complexes.[23]

2.2.9. Pharmacokinetic Analysis and Drug-likeness Prediction. The open-source program ADMET Lab 2.0 from computational biology and drug design group (https://admetmesh.scbdd.com/ accessed on November 19, 2023) was used to determine the ADMET parameters of aspartame, sucralose, sucrose, and sorbitol.[24]

2.2.10. Statistical Analysis. The statistical program SPSS/PC (version 22.0; SPSS Inc., Chicago, IL, USA) was employed to conduct one-way analyses of variance (ANOVA) and post hoc multiple comparisons using the Duncan test.

3. RESULTS AND DISCUSSION

3.1. In Vivo Study Results. *3.1.1. Pharmacological, Toxicological, and Clinical Observations.* Artificial sweeteners are safe for human consumption in accordance with the ADI limit, according to the FDA and EFSA as aspartame dose is 50 mg/kg/day, sucralose dose is 5 mg/kg bw/day, sucrose dose is 40 mg/kg bw/day, and the normal level of consumption of sorbitol is 25 g daily in two doses.[20] Nevertheless, there is a lack of explicit safety assertions about sweeteners' impact on noncommunicable disorders. Some concerns still exist regarding the impact of unmetabolized compounds on the hormone levels. Throughout the present investigation, there were no observable alterations in behavior and no evident manifestations of significant system failure, such as diarrhea, salivation, oliguria, etc.

3.1.2. Effect of Natural and Artificial Sweeteners on Water Consumption. Figure 1 summarizes the daily ingestion of water data for all of the experimental groups. Average daily water consumption during the experiment showed increased intake in all groups (aspartame, sucralose, sucrose, and sorbitol) compared to the normal control group. The water consumption was statistically significantly increased, especially in the aspartame group, then sucralose group during the 12 weeks, as this could be attributed to its correlation with the intensely sweet flavor of aspartame and its hedonic effect. Furthermore, the sucrose group, which used sucrose as a natural sweetener, exhibited considerably greater fluid consumption than the sorbitol group, which used sorbitol as an artificial sweetener. This disparity may be attributed to sucrose's more enjoyable and pure taste compared to sorbitol.[25,26]

The amount of sugar that the animal groups consumed was not controlled in our experiment, as the sweeteners were provided freely as drinking solutions. The rationale behind this was to emulate how consumers use these commercial sweeteners. Nevertheless, this is a constraint of our research as it is a comparative study.[26]

3.1.3. Effect of Natural and Artificial Sweeteners on the Nutritional Parameters. Using non-nutritive sweeteners in various commercial products is intended to facilitate their consumption and preserve the desirable sweet flavor that appeals to the general public while reducing calorie consumption. This phenomenon can be attributed to the growing incidence of overweight and obesity, which necessitates efforts to reduce and sustain body weight. The regulation of appetite is a complex process wherein hunger and satiety play crucial roles in regulating food consumption.[27] Studies conducted by Rosales-Gómez et al.[8] and Brown et al.[28] indicate that increased sweetness intensity is associated with a heightened liking for sweet flavors and appetite.

No significant alterations in initial body weights and total food intake were found in any of the groups compared to the normal control group, as indicated by the findings reported in Table 1. In the interim, it is noteworthy that all experimental groups exhibited substantial increases in final body weight, weight gain, and feed efficiency compared to the control group (Table 1). Data suggested that artificial sweeteners may not be suitable for weight gain management, especially in overweight or obese individuals. Also, our results agreed with a study by Rosales-Gómez et al.[8] who reported that artificial sweeteners raise weight and body mass index (BMI) in healthy, sedentary individuals. Similarly, Yang[29] reported a rise in BMI and body weight in healthy participants who drank liquids containing three distinct sweeteners over an extended period, as opposed to those who drank liquids without sweeteners. Also, a more significant increase was observed in the aspartame group compared to the normal control group. Investigations

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Table 3. Effect of Natural and Artificial Sweeteners on the Protein and Peptide Hormones[a]

groups	insulin (μLU/mL)	glucagon (pg/mL)	leptin (ng/mL)	amylin (pg/mL)	GIP (pmol/L)	GLP-1 (pmol/L)
normal control	17.63 ± 0.68	15.52 ± 0.23	10.65 ± 0.19	20.47 ± 0.29	12.47 ± 0.29	18.47 ± 0.29
aspartame group	9.43 ± 0.16[a]	7.45 ± 0.14[a]	14.58 ± 0.23[a]	8.45 ± 0.14[a]	8.77 ± 0.59[a]	23.43 ± 0.16[a]
sucralose group	10.93 ± 0.62[b]	8.82 ± 0.52[b]	13.28 ± 0.19[b]	10.82 ± 0.44[b]	9.73 ± 0.34[b]	23.07 ± 0.6[b]
sucrose group	12.02 ± 0.58[c]	9.42 ± 0.19[c]	12.77 ± 0.5[c]	11.35 ± 0.18[c]	10.35 ± 0.18[c]	22.35 ± 0.18[c]
sorbitol group	13.07 ± 0.80[d]	9.93 ± 0.6[d]	12.57 ± 0.24[d]	11.77 ± 0.42[d]	10.57 ± 0.38[d]	22.32 ± 0.13[d]

[a]The values were shown as mean ± SD (n = 6), where different letters indicate a significant difference at $P < 0.05$, and the same letters in each column indicate a nonsignificant difference between categories.

conducted by de Matos Feijó et al., Choudhary and Devi and Bian et al.[30−32] have shown that feeding rats and mice aspartame enhances their appetite and causes them to acquire weight. This result may be attributed to the indirect inhibition of intestinal alkaline phosphatase (IAP) by phenylalanine (PHE), a metabolite of ASP, thus promoting the elevation in body weight gain.[33] Phenylalanine can affect appetite by releasing cholecystokinin and stimulating hypothalamic adrenoreceptors. Aspartate metabolite is taken up by the brain's ARC nucleus, which synthesizes neuropeptide Y that stimulates carbohydrate intake.[27]

3.1.4. Effect of Natural and Artificial Sweeteners on the Hematological Parameters. Hematological indicators offer an excellent foundation for assessing the presence of disease, the degree of tissue destruction, the reaction of the antioxidant defense mechanism, and the overall state of health. The data collected on the rats were within their physiological norm, which was appropriate for their age. Table 2 showed that the results revealed the hematological profiles of rats for all experimental groups. Compared with the normal control group, the results revealed a notable increase in the blood counts of RBC and platelets. The utilization of the mean RBC size has facilitated the enhanced characterization of anemia and the quantification of RBC dimensions within the circulatory system. Platelets are blood components that aid in clotting. It is plausible that the elevated blood parameters seen in the experimental rats may be associated with the oxidative damage likely induced by the various sweeteners.

Furthermore, all experimental groups noticed a significant reduction in the level of WBC, Hb, and Hct. The decrease in WBC counts results from cell redistribution to injured organs, including the liver, as opposed to a loss of cells.[19,27] In addition, the notable increase in RBCs and decrease in hemoglobin (Hb) levels observed in all experimental groups can be attributable to macrocytic anemia, a hematological condition characterized by the aberrant production of disproportionately sizable RBCs by the bone marrow. Although macrocytic anemia is not a grave condition, it can lead to significant medical complications if not promptly addressed. Macrocytic anemia arises from insufficient vitamin B12 and folate (vitamin B9) intake, resulting in impaired erythropoiesis or impaired absorption of these essential nutrients due to medical disorders. In our study, macrocytic anemia was developed because we have an underlying condition that arose from the consumption of natural and artificial sweeteners for 12 weeks, and this result was agreed with some studies.[34−36]

When the body is provided with sweeteners, it undergoes a series of sensory cues that aid in the preparation for metabolic digestion and use. So, based on the findings of hematological investigations, we observed that the aspartame group had a bad influence on blood morphological indicators, where the sorbitol group had a less noticeable effect. This result is agreed with other studies.[37−40]

3.1.5. Effect of Natural and Artificial Sweeteners on the Different Hormones and Inflammatory Cytokines. The results presented in Table 3 showed a significant decrease in insulin, glucagon, amylin, and GIP levels in all experimental groups (aspartame, sucralose, sucrose, and sorbitol) compared to the normal control group. On the other hand, a significant elevation was noticed in leptin and GLP-1 levels in all experimental groups (aspartame, sucralose, sucrose, and sorbitol) compared to the normal control group.

The stimulation of insulin secretion occurs when natural sugars and artificial sweeteners engage with sweet-taste receptors in the pancreatic β-cells. This interaction triggers a signal transduction cascade through a mechanism that relies on Ca^{2+} and cAMP.[41] This observation implies that artificial sweeteners may elicit a lower insulin secretion level than natural sugars.[42] In our investigation, we found that sucralose can decrease insulin secretion. This finding is consistent with a previous study conducted by Gupta et al.[43] which demonstrated that sucralose has been implicated in developing a prediabetic condition. The research findings indicate that the use of sucralose results in substantial harm to the pancreas, resulting in the deterioration of its structural integrity and the loss of its islets and β cells. Chronic exposure to sucralose may result in an initial increase in insulin secretion, followed by a subsequent decrease in insulin production due to the depletion of insulin secretory granules. This ultimately leads to a loss of first-phase insulin secretion. An impairment in insulin secretion during the early stages is an early indicator of the development of type 2 diabetes mellitus.[44,45]

Our study's data also indicates that various sweeteners uniquely impact releasing hormones in the pancreas and gastrointestinal tract. Due to the simultaneous release of amylin and insulin in a 1:100 ratio, the response profile was anticipated to closely resemble that of insulin, as seen in this study. While amylin is acknowledged for its ability to decelerate stomach emptying, hence promoting satiety, alternative research has indicated that it could potentially harm pancreatic β-cells.[46,47] Amylin is susceptible to spontaneous post-translational changes, resulting in the buildup of amyloid plaques in the pancreatic islets and the subsequent malfunctioning of β-cells. Furthermore, reports indicate that amylin is associated with developing insulin resistance in skeletal muscle. Hence, it is plausible that long-term consumption of both natural and artificial sweeteners is linked to reduced amylin responses.[48]

Incretin hormones are an additional component that play a role in maintaining homeostatic systems involved in regulating glucose metabolism. The aforementioned hormones are peptides secreted by enteroendocrine cells in the small intestine. They are released in response to food consumption

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Table 4. Effect of Natural and Artificial Sweeteners on the Glycoprotein Hormones and Eicosanoid Hormone[a]

parameters	groups				
	normal control	aspartame group	sucralose group	sucrose group	sorbitol group
FSH (mIU/ML)	9.47 ± 0.29	5.55 ± 0.21[a]	6.10 ± 0.64[b]	6.35 ± 0.18[c]	3.73 ± 0.45[d]
LH (mIU/ML)	5.25 ± 0.14	2.37 ± 0.18[a]	2.60 ± 0.18[b]	2.72 ± 0.30[c]	2.80 ± 0.36[d]
TSH (mIU/ML)	2.1 ± 0.09	5.8 ± 0.47[a]	5.2 ± 0.88[b]	4.9 ± 1[c]	4.7 ± 0.98[d]
PTH (pg/mL)	200.47 ± 0.29	220.43 ± 0.16[a]	218.93 ± 0.51[b]	217.75 ± 0.1[c]	216.8 ± 0.9[d]
prostaglandin E2 (pg/mg tissue)	13.65 ± 0.19	31.58 ± 0.23[a]	29.95 ± 0.5[b]	29.27 ± 0.16[c]	28.57 ± 0.24[d]

[a]The values were shown as mean ± SD (n = 6), where different letters indicate a significant difference at $P < 0.05$, and the same letters in each row indicate a nonsignificant difference between categories.

Table 5. Effect of Natural and Artificial Sweeteners on the Steroid and Amine Hormones[a]

group	testosterone (ng/dL)	progesterone (pg/mL)	estradiol (pg/mL)	T3 (ng/dL)	T4 (μg/dL)	epinephrine (ng/mL)	norepinephrine (ng/mL)
normal control	1.47 ± 0.29	2.47 ± 0.29	31.07 ± 0.68	12.07 ± 1.75	3.23 ± 0.05	9.65 ± 0.19	6.15 ± 0.72
aspartame group	0.55 ± 0.21[b]	0.43 ± 0.16[b]	17.40 ± 0.23[b]	8.77 ± 0.43[b]	2.43 ± 0.16[b]	11.58 ± 0.23[b]	8.08 ± 0.5[b]
sucralose group	0.6 ± 0.18[c]	0.60 ± 0.18[c]	17.70 ± 0.49[c]	9.6 ± 0.18[c]	2.6 ± 0.18[c]	10.78 ± 0.56[c]	7.28 ± 0.19[c]
sucrose group	0.7 ± 0.14[d]	0.75 ± 0.1[d]	18.23 ± 0.16[d]	10.08 ± 0.58[d]	2.62 ± 0.23[d]	10.27 ± 0.16[d]	7.1 ± 0.32[d]
sorbitol group	0.83 ± 0.28[e]	0.80 ± 0.11[e]	18.50 ± 0.21[e]	10.17 ± 0.47[e]	2.68 ± 0.21[e]	10.23 ± 0.37[e]	7.07 ± 0.6[e]

[a]The values were shown as mean ± SD (n = 6), where different letters indicate a significant difference at $P < 0.05$, and the same letters in each column indicate a nonsignificant difference between categories.

and play a significant role in regulating satiety and maintaining the balance of plasma glucose levels. The metabolic activity of GIP was quantified.[50,51] The study's findings indicate that artificial sweeteners, specifically aspartame and sucralose, decrease the secretion of GIP without altering insulin levels. Additionally, these sweeteners were observed to increase the body weight despite decreased feed intake. This effect is likely attributed to increased leptin secretion, as the artificial sweeteners enhanced the secretion of GLP-1. These results are consistent with previous studies conducted by Parker et al., Steinert et al., and Alshafei et al.[52−54]

Sweet taste receptors, which are heterotrimeric G-protein coupled receptors (GPRs) composed of two subunits, namely taste receptor type 1 member 2 (T1R2) and 3 (T1R3), are responsible for perceiving gustatory information following the consumption of either natural sugars or artificial sweeteners.[55,56] The taste receptors responsible for flavor perception are situated within taste buds in the oral cavity and extraoral regions, such as the gut and pancreatic β-cells.[57] The binding sites of artificial sweeteners and natural sugars differ in sweet taste receptors.[29,58] When sweet substances engage with the sweet receptor T1R2/T1R3, the heterotrimeric G protein a-gustducin is activated.[59]

Enteroendocrine L-cells release glucagon-like peptide-1 (GLP-1) when natural sugars connect to sweet taste receptors, while K-cells release a glucose-dependent insulinotropic peptide (GIP). The hormones can traverse the semipermeable blood−brain barrier (BBB), thereby accessing the hypothalamus and influencing food consumption by reducing appetite and promoting fullness. Nevertheless, artificial sweeteners may not exhibit the same potency as natural sugars in secreting GLP-1 and GIP in vivo, as their secretion is contingent upon nutritional availability.[60] Aspartame is digested and absorbed before reaching the lower gastrointestinal tract, where it binds to the receptors responsible for the sweet taste. Sucralose and sorbitol traverse the lower gastrointestinal system to undergo absorption, digestion, or direct elimination. In addition, natural sugars can increase the secretion of incretins, hence promoting insulin secretion by β-cells. However, it is important to note that artificial sweeteners do not directly trigger incretin secretion, as this process seems dependent on food availability.[42]

There is still some debate over the effects of natural and artificial sweeteners on human health. The results presented in Table 4 showed that hormones, including FSH and LH, are significantly decreased in all groups (aspartame, sucralose, sucrose, and sorbitol) compared to the normal control group, while TSH, PTH, and prostaglandin E2 hormones are elevated in all groups (aspartame, sucralose, sucrose, and sorbitol) compared to the normal control group.

We observed that the aspartame and sucralose groups had a noticeable negative influence on FSH and LH hormones. The reduction in both FSH and LH hormone levels is an indicator of a disorder known as hypogonadotropic hypogonadism. Insufficient activation of the Leydig cells, caused by defects in the hypothalamus or pituitary, leads to low testosterone levels. This finding is supported by the data presented in Table 5. Insufficient concentrations of these hormones may contribute to diminished sperm counts, hence constituting a primary factor contributing to male infertility. Secondary hypogonadism patients can regain their fertility by appropriate hormonal stimulation, while individuals with original hypogonadism caused by testicular failure are unable to do so. The findings presented in this study are consistent with a previous investigation conducted by Azeez,[17] which demonstrated that extended exposure to aspartame in white Wistar rats resulted in neurodegenerative effects, specifically in the hypothalamus. Additionally, the study revealed notable alterations in the structure and function of the hypothalamic−pituitary axis. The altered hormone concentrations led to a decrease in the production of LH and FSH, as well as the inhibition of testosterone synthesis and release. Consequently, this resulted in a decline in the reproductive capacity.[49]

There is a correlation between the consumption of artificial sweeteners, such as aspartame and sucralose, and an increase in TSH levels. Elevated levels of TSH are regarded as a reliable indicator of a specific form of hypothyroidism known as Hashimoto's thyroid disease (HT). In a study by Helal et al.[61]

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Table 6. Effect of Natural and Artificial Sweeteners on Inflammatory Cytokines[a]

	groups				
parameters	normal control	aspartame group	sucralose group	sucrose group	sorbitol group
TNF-α (pg/mL)	56.48 ± 0.47	179.08 ± 4.21[a]	154.95 ± 1.75[b]	138.6 ± 40.44[c]	137.9 ± 6.16[d]
IFN-γ (μg/mL)	57.48 ± 2.71	95.42 ± 3.11[a]	85.28 ± 3.2[b]	80.43 ± 5.2[c]	75.4 ± 3.1[d]
IL-1 (pg/mL)	28.32 ± 1.68	75.47 ± 3.09[a]	72.12 ± 1.88[b]	70.93 ± 4.49[c]	66.07 ± 2.79[d]
IL-6 (pg/mL)	37.82 ± 1.86	78.42 ± 4[a]	77.12 ± 3.43[b]	74.93 ± 1.86[c]	70.07 ± 3.21[d]
IL-10 (pg/mL)	59.15 ± 2.08	87.25 ± 2.36[a]	80.62 ± 5.91[b]	78.6 ± 4.29[c]	77.9 ± 1.63[d]
IL-1β (pg/mg protein)	89.15 ± 2.01	157.08 ± 2.37[a]	152.57 ± 5.16[b]	146.1 ± 1.92[c]	142.73 ± 3.51[d]

[a]The values were shown as mean ± SD (n = 6), where different letters indicate a significant difference at $P < 0.05$, and the same letters in each row indicate a nonsignificant difference between categories.

two of every three individuals who eventually ceased using artificial sweeteners experienced a complete reversal of their hyperglycemia. The thyroid antibodies exhibited a steady restoration to their baseline levels, and subsequently, cessation of hormone replacement therapy was achieved. This response supports the notion that the cessation of sweets may be potentially associated with thyroid illness.

The acronym PTH denotes parathyroid hormone. Cysteine is a protein hormone secreted by the parathyroid gland, consisting of four small glands situated on or close to the thyroid gland in the cervical region. PTH regulates calcium, phosphorus, and vitamin D concentrations in the bloodstream, which is significant for bone development. According to our results, a high PTH level results in hyperparathyroidism, parathyroid gland tumor, kidney disease, or a vitamin D deficiency.[62,63]

Prostaglandins encompass various distinct categories and serve crucial functions in regulating biological functions such as inflammation, pain, and uterine contractions. The elevated levels of prostaglandins seen in this study could be attributed to either a suppression in DNA production in the testes or a reduced responsiveness of the receptors to high concentrations of prostaglandins.[61,64]

The results presented in Table 5 showed that hormones including testosterone, progesterone, estradiol, T3, and T4 are significantly decreased in all groups (aspartame, sucralose, sucrose, and sorbitol) when compared to the normal control group where epinephrine and norepinephrine hormones are elevated in all groups (aspartame, sucralose, sucrose, and sorbitol) compared to the normal control group.

Consuming artificial sweeteners can result in a range of thyroid dysfunctions. Endocrine disorders resulting from the intake of sweeteners are frequently linked to a diverse array of adverse outcomes. The impact of artificial sweeteners, particularly those belonging to the aspartame and sucralose families, on the thyroid hormonal balance has been empirically demonstrated, mainly through the activation of hepatic catabolism. The results of our study validate the findings of Pałkowska-Goździk et al.[65] who examined the thyroid functioning condition in rats following 3 weeks of sucralose use. The researchers observed that the consumption of sucralose resulted in alterations in thyroid peroxidase activity, subsequently leading to a decrease in the production of thyroid hormones. Modifications in the peripheral metabolism of thyroid hormones may be a potential underlying factor for this phenomenon.[65] The study's findings indicate that sucralose exhibits metabolic activity and can potentially exacerbate metabolic diseases by negatively impacting thyroid hormone metabolism.[66] The correlation between Hashimoto's illness and the excessive intake of sugar substitutes was established through the observation of a swift restoration of thyroid hormone and thyroid antibody levels to their normal range after the cessation of sucralose consumption.[67]

The presence of androgens is crucial for the preservation and advancement of sexual function in males. The HPG axis is critical for regulating testosterone production in eugonadal males. The release of GnRH from the hypothalamus and LH secretion from the pituitary gland is inhibited by testosterone, reducing both frequency and amplitude. The Sertoli cells of the testes not only stimulate spermatogenesis but also emit the glycoprotein hormone inhibin. This hormone acts as a negative feedback mechanism to the pituitary gland, decreasing the secretion of FSH. In target cells, testosterone undergoes conversion to dihydrotestosterone (DHT) through the action of 5β-reductase enzymes or to estradiol through aromatase. The pharmacological and physiological effects of testosterone and dihydrotestosterone (DHT) are exerted by binding to the androgen receptor.[68,69]

Progesterone is a building block for many hormones including testosterone. Low levels can result in a lack of sex drive, and imbalance can cause "estrogen dominance", leading to several conditions like erectile dysfunction, fatigue, and low libido. Insufficient progesterone levels can arise from various circumstances, such as weight increases and an unhealthy diet. However, it is essential to note that hypothyroidism is also a contributing factor,[70−72] as seen by the findings presented in Table 4.

E2, or estradiol, is a vital estrogen hormone for both men and women. In men, estradiol is a minor hormone that plays a role in male sex hormone physiology. It is synthesized from testosterone and androstenedione and regulates bone density, sexual function, and cognitive function, among other things. Insufficient amounts of erythropoietin (E2) in males can give rise to a range of symptoms, encompassing diminished sexual desire, erectile dysfunction, diminished muscle mass and strength, the process of aging, obesity, and specific medical ailments such as hypogonadism.[73,74]

The thyroid gland produces hormones and is part of the hypothalamic-pituitary-thyroid axis. The hypothalamus secretes thyroid-releasing hormone (TRH) as part of the axis. The release of TSH from the anterior pituitary gland is stimulated by thyroid-relative hormone (TRH). TSH induces the thyroid gland to produce thyroxine (T4) and triiodothyronine (T3), which exist in two forms: free, active, and bound, inert. Thyroid abnormalities primarily result from intrinsic thyroid malfunction, with secondary causes, such as a pituitary adenoma secreting TSH, pituitary failure, or hypothalamic failure, being infrequent. TSH is, therefore, often elevated,[61] while T3 and T4 levels are generally low in hypothyroidism (Tables 4 and 5).

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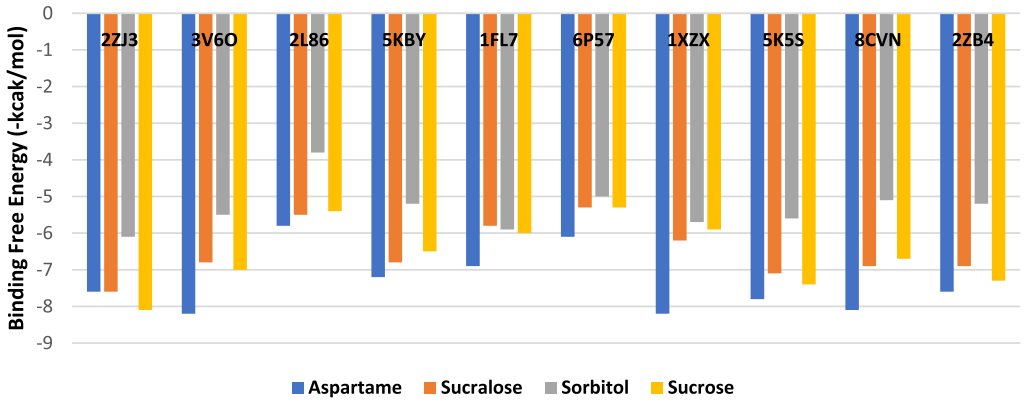

Figure 2. Molecular docking analysis of sweeteners on target proteins and receptors associated with proteins, peptides, glycoproteins, and eicosanoid hormones.

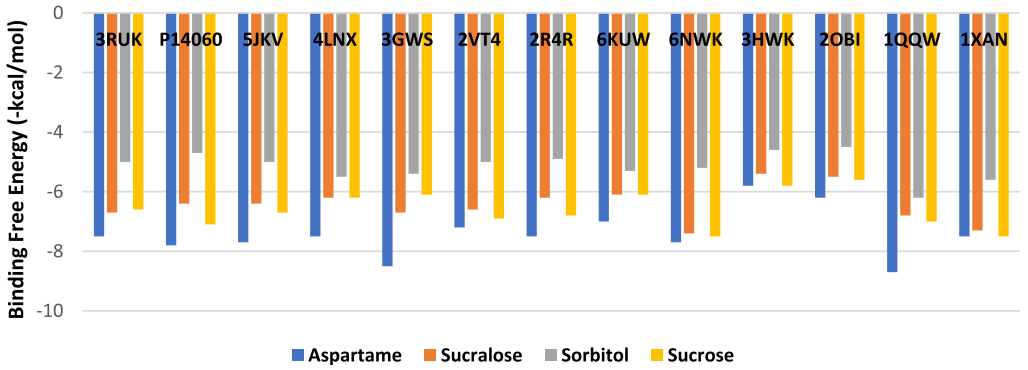

Figure 3. Molecular docking analysis of sweeteners on target proteins and receptors associated with steroid and amine hormones and inflammatory cytokines.

The activation of alpha- and beta-adrenoreceptors in multiple organs and tissues, such as the heart, lungs, muscles, and blood vessels, is induced by epinephrine. Numerous physiological changes, including elevated blood sugar levels, faster breathing, increased heart rate and blood flow, and enhanced strength and athletic performance, are brought on by the release of adrenaline into the bloodstream.[75,76] Norepinephrine can increase the blood pressure and heart rate. Excess production due to medical conditions like stress, obesity, and tumors can cause symptoms such as sweating, irregular heartbeat, high blood pressure, and headaches. High levels can also increase the risk of cardiovascular and kidney damage.[77]

Cytokines regulate physiological processes in the testis, such as germ cell development, Leydig cell steroidogenesis, and extracellular matrix production.[78,79] According to results presented in Table 6, aspartame, sucralose, sucrose, and sorbitol groups had significantly higher levels of all anti- and pro-inflammatory cytokines, including TNF-α, IFN-γ, IL-1, IL-6, IL-10, and IL-1β compared to the normal control group. That because the prolonged consumption of both natural and artificial sweeteners over 12 weeks caused an elevation in oxidative stress, producing elevated levels of reactive oxygen species (ROS), upregulation of pro-apoptotic proteins, decreased antioxidant levels, deterioration of mitochondrial function, and triggered the release of pro-inflammatory cytokines that regulate immune responses. These disruptions are associated with structural and functional changes that initiate an instantaneous inflammatory response, leading to tissue destruction. These effects potentially impacting the integrity of Sertoli tight junctions, attachment of germ cells, and cell death of germ cells. The research conducted by Calder et al[80] and Soliman et al.[81] corroborated our findings by indicating that the inflammatory response triggered by different substances is attributed to elevated levels of inflammatory cytokines, such as TNF- α, IL-1, IL-6, and IL-1β. This reaction encompasses a multitude of processes, cells, and molecules. Irrespective of the underlying cause, these indications are consistently observed in all instances of inflammation.[82,83] The deficiency in sex hormones reported in Table 5 is correlated with the higher inflammatory cytokines discussed and revealed in Table 6, as reported by Mostafa Mohammed et al.[84]

3.2. Molecular Docking Study. To further explore the effect of different sweeteners under investigation on the protein and peptide, glycoprotein, eicosanoid, steroid, and amine hormones, in addition to the inflammatory cytokines, the in silico molecular docking technique was employed to evaluate the sweeteners' binding affinities and binding manner at the active sites of various target proteins associated with the hormones under investigation (Figures 2 and 3). The CB-DOCK software, known for its reproducible conformations and accuracy in molecular docking and scoring, was employed as a molecular docking tool to examine the conformations of the candidate compounds with target proteins. The compounds exhibit a favorable and stable binding posture within the binding pocket of the target protein, as shown by their very low energy scores (ΔG).

Based on the idea that a more negative score indicates a better affinity, as shown in Figure 2, the three binding ligands,

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aspartame, sucralose, and sucrose, exhibited higher docking scores to the receptors associated with the actions of protein, peptide, glycoprotein, and eicosanoid hormones compared to sorbitol. For example, aspartame showed remarkable scores ranging from −7.6 to −8.2 kcal/mol on docking with the receptor proteins associated with insulin resistance, leptin receptor, TSH receptor, human calcium-sensing receptors, 15-hydroxyprostaglandin dehydrogenase (PDB ID: 8CVN), and 15-keto prostaglandin-13-reductase (PDB ID: 2ZJ3, 3V6O, 1XZX, 8CVN, 5K5S, and 2ZB4). In comparison, the highest binding affinities for sucrose and sucralose were found with PDB ID: 2ZJ3 as −8.1 and −7.6 kcal/mol, respectively. The remaining binding free energy values between sweeteners and other receptors were still noticeable but lower, which revealed a lower effect.

It was predictable to find a higher binding affinity among the sweeteners investigated in the current study, and GFAT (PDB ID: 2ZJ3), which is responsible for insulin resistance with −6.1 to −8.1 kcal/mol (Figure 2), in agreed with Damián-Medina et al.[85] for phenolic compounds and Mohammed et al.[23] for food flavorings. The study found that the baseline GLP-1 sensitivity was 3- to 4-fold higher than that of GIP. It is believed that the glucose-lowering effect of DPP-4 inhibitors is mainly due to the action of GLP-1, as the GIP receptor is downregulated under hyperglycemic conditions.[86] Therefore, the significant increase in GLP-1 observed, especially for aspartame and sucralose groups, could be associated with their inhibition effect on DPP IV (PDB ID: 5KBY) with binding free energy: −7.2 and −6.8 kcal/mol for aspartame and sucralose (Figure 2).

Blocking or stimulating the leptin receptor, responsible for transferring leptin across the BBB, is a major pharmacological approach to inhibit or stimulate leptin's activity in the hypothalamus and the periphery.[87] Both aspartame (−8.2 kcal/mol) and sucrose (−7.0 kcal/mol) showed the highest affinity toward leptin receptor (PDB ID: 3V6O), followed by sucralose (−6.8 kcal/mol), as shown in Figure 2. The previous findings agree with body gains observed in Table 1, which revealed sweeteners' blocking or antagonistic effect against leptin receptors.

The in vivo results of the present study agree with many published studies on inhibiting human amylin aggregation. For example, the flavonoid chrysin inhibited amylin through in silico and in vitro approaches, with a binding free energy of −6.45 kcal/mol.[88] Also, Fang et al.[89] revealed amylin inhibition using epigallocatechin-3-gallate (−4.28 kcal/mol) and genistein (−5.10 kcal/mol). In the present study, the docking study with amylin (PDB ID: 2L86), aspartame (−5.8 kcal/mol), sucralose (−5.5 kcal/mol), and sucrose (−5.4 kcal/mol) showed comparable results to the literature (Figure 2), which may the cause for the lower concentrations of amylin as shown in Table 3.

In a study conducted by Bhogireddy et al.[90] molecular docking techniques were employed to assess the structure of FSH (PDB ID: 1XWD) against the ZINC database, which contains 2.7 million compounds. The analysis was conducted using known standard compounds as a reference. The findings of this study suggest that these compounds may be further evaluated for their potential to inhibit FSH. In agreeing with the literature cited above, the findings of the present study showed lower concentrations of both FSH and LH hormones, as shown in Table 4, due to the docking interaction revealed in Figure 2 between the examined sweeteners and FSH hormone (PDB ID: 1FL7) and LH hormone (PDB ID: 6P57), where aspartame has the highest binding affinities toward both; −6.9 and −6.1 kcal/mol, respectively.

Due to the significant increase in PTH levels in rats' groups with sweeteners (Table 4), it was important to examine the docking between the sweeteners under investigation in the current study and human calcium-sensing receptor extracellular domain (PDB IDs: 5K5S) as shown in Figure 2. Aspartame showed the highest affinity against the receptors with −7.8 kcal/mol, followed by sucrose (−7.4 kcal/mol) and sucralose (−7.1 kcal/mol), which caused the rise in PTH levels. The previous findings agreed and are higher than the docking scores recorded by tenofovir disoproxil fumarate as an antiretroviral agent on the same receptor with −7.35 kcal/mol.[91]

Many studies have reported the effect of different natural and synthetic compounds as COX I/II suppressors or anti-inflammatory agents that could inhibit the production of prostaglandin E2.[92] The activation of two key catabolic enzymes, 15-hydroxyprostaglandin dehydrogenase (PDB ID: 8CVN), and 15-keto prostaglandin-13-reductase (PDB ID: 2ZB4), can essentially eliminate the biological activity of prostaglandin E2.[93] However, aspartame showed the highest affinity toward both enzymes with −8.1 and −7.6 kcal/mol, followed by sucrose and sucralose, while sorbitol was the least (Figure 2), which revealed the avoidance of prostaglandin E2 suppression and, consequently, presence in higher concentrations compared to the control.

The binding affinities of aspartame to the thyroid receptors and TSH (from −7.5 to −8.5 kcal/mol) were higher compared to ethanoamide, which has affinity bonds with the TRβ receptors, 1Q4X and 3JZC, as −7.283 and −7.243 kcal/mol, respectively.[94] In the same line, the interactions of the three major polyphenols of *Ficus religiosa* L. with TSHR showed comparable binding affinities to the results of the present study with −8.2 kcal/mol for betulinic acid, followed by −7.8 kcal/mol for chlorogenic acid, and −5.9 kcal/mol for quinic acid.[95] Such higher binding affinity toward TSHR, induced hypothyroid states resulted in higher TSH and lower T3 and T4 levels,[61] as shown in Tables 4 and 5.

Docking of aspartame with the receptor proteins associated with the actions of steroid and amine hormones showed the highest scores among the tested sweeteners, ranging from −7 to −8.5 kcal/mol, which aligns with its effect on these hormones revealed by the in vivo study. The highest scores were against the T4 receptor with PDB ID: 3GWS (−8.5 kcal/mol) and human 3β-hydroxysteroid dehydrogenase with PDB ID: P14060 (−7.8 kcal/mol). In agreeing with the docking score of aspartame on glucocorticoid receptor with PDB ID: 6NWK (−7.7 kcal/mol), both sucrose and sucralose showed the highest energy against the same receptor with −7.5 and −7.4 kcal/mol. The negative inflammation activity of sweeteners showed by inflammatory cytokines determined in vivo is revealed by the highest docking scores of aspartame against antioxidant enzymes that resist oxidative stress, especially glutathione reductase (PDB ID: 1XAN) and catalase (PDB ID: 1QQW) with the scores −7.5 and −8.7 kcal/mol. Also, the highest scores by sucrose and sucralose were against 1XAN: −7.5 and −7.3 kcal/mol (Figure 3).

Through a molecular docking study, Gomes et al.[96] rerank score values and demonstrate that the three compounds extracted from Eleutherine plicata herb favorably interact with antioxidant enzymes, developing a receptor−ligand complex.

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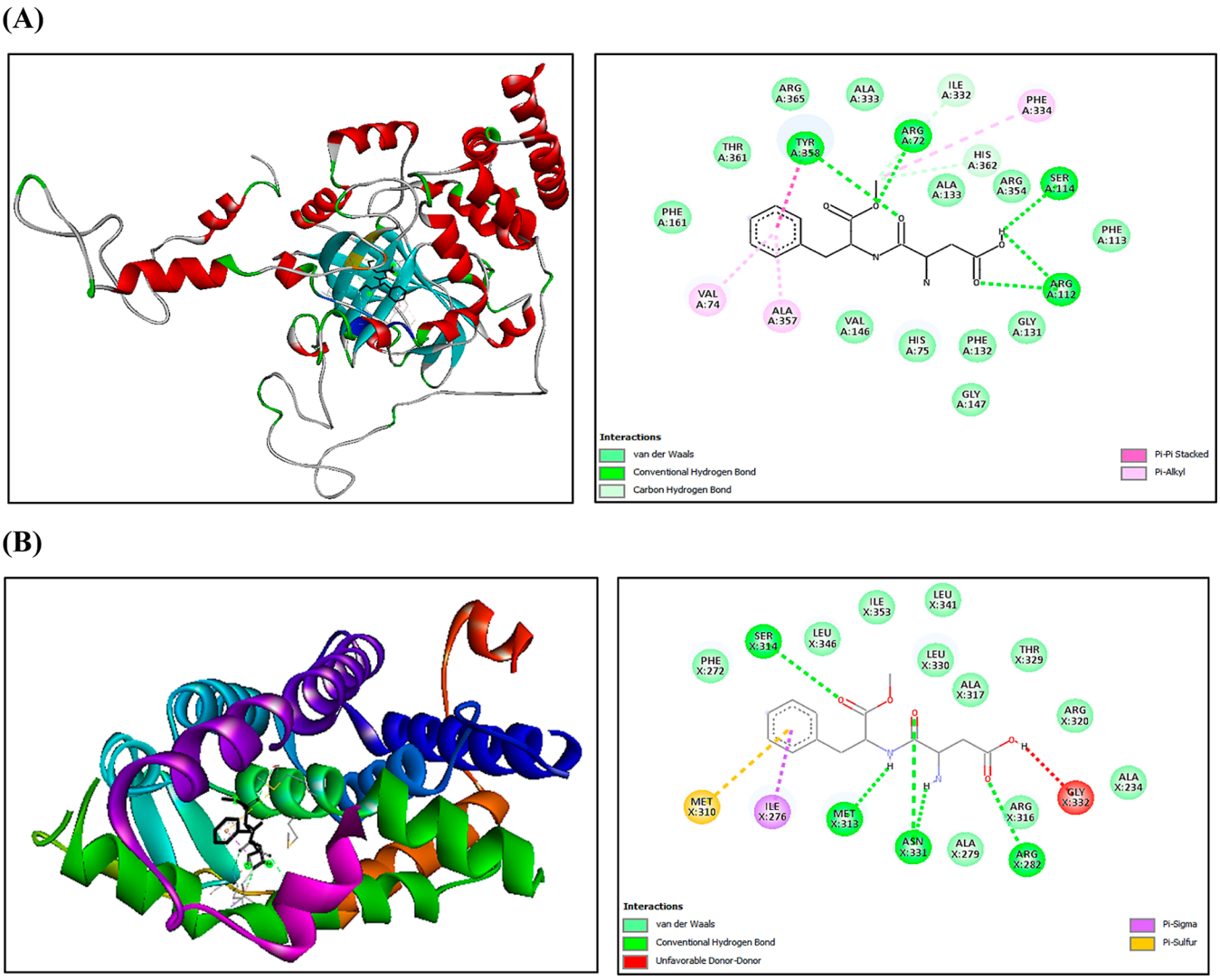

Figure 4. Interactions of Aspartame with Catalase PDB: 1QQW (A) and human thyroid hormone receptors β PDB: 3GWS (B).

This suggests that these molecules can suppress the activity of these proteins. Similarly, in the present study, sweeteners, especially aspartame, showed the highest docking capacity with catalase enzyme (PDB ID: 1QQW): −8.7 kcal/mol, glutathione reductase (PDB ID: 1XAN): −7.5 kcal/mol, glutathione peroxidase 4 (PDB ID: 2OBI): −6.2 kcal/mol, and superoxide dismutase (PDB ID: 3HW7): −5.8 kcal/mol (Figure 3). The remaining sweeteners, sucralose, sorbitol, and sucrose, also showed a remarkable binding affinity with the examined antioxidant enzymes but lower than aspartame. The previous findings are aligned with the increase in pro- and anti-inflammatory cytokines reported in Table 6.

Adrenergic receptors mediate the actions of epinephrine and norepinephrine. β-blockers such as propranolol and atenolol are widely used for medical conditions, including hypertension, heart failure, chest pain, migraines, and anxiety. In this study, sweeteners binding to the β-adrenergic receptors act as selective antagonist-blocking agents, especially aspartame, with the highest free docking energies (from −7.0 to −7.5 kcal/mol). This result agreed with the findings observed in Table 5.

Li et al.[97] showed binding scores ranging from −6.1 to −8.3 kcal/mol between the following inhibitors: triclosan, triflumizole, dichlone, and oxine and human placental HSD3B1 and rat placental HSD3B4, which are involved in the production of progesterone in the placenta and are essential for maintaining pregnancy. In agreement with the literature, the investigated sweeteners of this study showed noticeable docking scores with human 3β-HSD (PDB: P14060) ranging from −7.8 kcal/mol for aspartame to −7.1 kcal/mol for sucrose, −6.4 kcal/mol for sucralose, and finally −4.7 kcal/mol for sorbitol (Figure 3), which constitutes potential inhibition for progesterone and represents a fatal danger in pregnancy.

According to the progesterone deficiency reported above, a consequent decrease in androgens or testosterone could be observed in the rats' groups with sweeteners, especially aspartame (Table 5). Again, the deficiencies in androgen coincide with the higher binding affinities between aspartame (−7.5 kcal/mol), sucralose (−6.7 kcal/mol), sucrose (−6.6 kcal/mol), and cytochrome P450 17A1 (PDB ID: 3RUK), as shown in Figure 3. Cytochrome P450 17A1 is an enzyme responsible for the production of androgens in humans. Androgen steroids often influence the growth of prostate cancer cells, and a novel approach to hindering androgen synthesis involves the inhibition of CYP17A1. This approach can help treat lethal metastatic castration-resistant prostate

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cancer. According to Mori Sequeiros García et al.[98] Cisplatin can inhibit testosterone synthesis by acting on P450scc. In another study, DeVore and Scott[99] obtained X-ray crystal structures of CYP17A1 in the presence of abiraterone, a steroidal inhibitor that the Food and Drug Administration has approved for late-stage prostate cancer, and TOK-001, an inhibitor that is currently undergoing clinical trials.

Estrogen blockers reduce estrogen levels in the body by stopping its production or limiting its effects. They can be natural or pharmaceutical. Sweeteners in the present study showed a higher affinity toward human placental aromatase cytochrome P450 (PDB ID: 5JKV), where aspartame was potential with −7.7 kcal/mol against both receptors, followed by sucrose (−6.7 kcal/mol) and sucralose (−6.4 kcal/mol) as shown in Figure 3. With the lower estradiol concentrations detected in the examined groups compared to the control (Table 5) sweeteners used in the current study are estrogen mimics or antiestrogens. Prafulla and Lata[100] conducted a study to reduce estrogen levels by inhibiting estrogen biosynthesis. The researchers developed innovative flavones and flavonoids that specifically targeted the active binding sites of aromatase, namely, Leu477 and Ser478. These compounds were subjected to molecular docking simulations using the PDB ID: 3EQM. The docking programs confirmed active selection by seeing that the designed flavonoids filled the same binding pocket. The docking scores varied between −6.9 and −8.9 kcal/mol, offering valuable insights into designing aromatase inhibitors based on their structure. Similarly, Shah et al.[101] assessed the binding relationship between flavonoid compounds and cytochrome enzymes while also examining the ADME/T characteristics of the compounds with the highest scores. The findings suggest that these compounds have the potential to serve as lead compounds in the development of novel aromatase inhibitors for the treatment of breast cancer.

Molecular docking is a widely used method in drug discovery. It helps identify novel compounds and predict ligand-target interactions. Its applications in drug discovery have evolved over the years, including prediction of adverse effects, drug repurposing, and target profiling. Additionally, according to the present study's findings, the potential clinical implications of these binding interactions in the context of consumption and metabolic health of many additives, such as sweeteners, could be predicted and ensured by both in vitro and in vivo studies. Based on the literature and in agreement with the above results, many natural and synthetic compounds showed a potential inhibition effect against many hormones and enzymes during consumption, such as amylin,[88,89] FSH and LH,[90] COX I/II,[92] prostaglandin E2,[93] and antioxidant enzymes.[96] The current study's findings open the perspectives toward using in silico techniques to predict the negative implications of many food additives, including sweeteners.

Figure 4A,B shows the interaction of the potent aspartame, revealing the highest docking scores with the crystal structures of catalase (PDB ID: 1QQW) and human thyroid hormone receptors β (PDB ID: 3GWS). The higher binding affinity of aspartame with 1QQW (−8.7 kcal/mol) is attributed to the conventional hydrogen bonding formed from the NH-group of ARG A:72 and 112 (H-donor accepted by the methoxy and carbonyl groups of aspartame), hydroxyl group of TYR A:358 (H-donor accepted by the carbonyl group of the aspartame), OH-group of aspartame (H-donor accepted by the carbonyl group of ARG A:112 and the OH-group of SER A:114), and finally the intramolecular bonds inside the ligand between the NH-group (H-donor) and the methoxy and carbonyl groups (H-acceptors) as shown in Figure 4A. Also, Carbon−Hydrogen interactions were observed between the methoxy groups of aspartame as H-donor and the carbonyl groups of ILE A:332 and NH-group of HIS A:362 as H-acceptors. In addition, $\pi-\pi$ stacked and π-alkyl hydrophobic interactions were observed between aspartame and 1QQW moieties and vice versa. The $\pi-\pi$ stacked interaction refers to the attractive force between aromatic rings. This is due to π-electron clouds such as the π-orbitals of TYR A:358 and aspartame (Figure 4A). Again, hydrophobic interactions between the π-orbitals of PHE A:334 and HIS A:362 and the alkyl groups of aspartame and conversely between the π-orbitals of aspartame and alkyl groups of VAL A:74 and ALA A:357 were observed (Figure 4A).

Conventional hydrogen bonding and hydrophobic $\pi-\sigma$ and π-sulfur interactions seem responsible for the high docking score of aspartame against 3GWS, as shown in Figure 4B. NH2-group of ARG A:282, OH-group of SER A:314, NH2-group of ASN A:331, and NH- and NH2-groups of aspartame as H-donors interacted conventionally with the carbonyl group of the aspartame, MET A:331, and ASN A:331 (Figure 4B). The $\pi-\sigma$ and π-sulfur hydrophobic interactions from the C−H of ILE A:276 to the π-orbitals of aspartame and from the sulfur of MET A:310 to the π-orbitals of aspartame. Finally, unfavorable donor−donor interaction was observed between the GLY A:332 amino group AS H-donor and the hydroxyl group of aspartame as also H-donor (Figure 4B).

Ferreira de Freitas and Schapira[102] discovered that N−H···O interactions, observed through a molecular docking study between aspartame and 1QQW, occurred more frequently than O−H···O and N−H···N interactions. Within the N−H···O interactions existed about equal quantities of hydrogen bonds that were either neutral or charged. More hydrogen bonds were observed in arginines than lysines, potentially attributed to the inclusion of three nitrogen atoms within the guanidinium group of arginine side chains. In biological complexes, hydrogen bonds serve as the predominant directional intermolecular interactions and substantially influence the specificity of molecular recognition. The magnitude of energy attributed to hydrogen bonding exhibits a range of −1.5 to −4.7 kcal/mol.[102]

When dealing with two π-systems, there are three common forms of interaction: T-shaped, edge-to-face, and parallel-displaced stacking arrangements. The interaction energies of these forms of interaction are as follows: −1.48 kcal/mol for parallel, −2.46 kcal/mol for T-shaped, and −2.48 kcal/mol for slipped-parallel benzene dimers.[103] Aromatic ring interactions are common in chemical and biological systems. They rank as the third most common hydrophobic interaction between proteins and ligands. The present study's conclusions are supported by the observation that over half of all stacking interactions occur between the aromatic ring of phenylalanine and an aromatic ring in the ligand. This is followed by tyrosine (36.8%), tryptophan (8.7%), and histidine (5.1%).[102] The π-alkyl interaction played a crucial role in the ligand's intercalation in the receptor's binding pocket.[104]

According to Ferreira de Freitas and Schapira,[102] a significant hydrophobic interaction observed in protein−ligand interactions pertains to the interaction between a sulfur atom originating from the side chain of methionine and an aromatic carbon derived from the ligand. According to a recent study conducted by Valley et al.[105] it has been observed that the

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Table 7. Computed ADMIT Properties of Sweeteners Using ADMETlab 2.0

properties*	compound			
	aspartame	sucralose	sorbitol	sucrose
Physicochemical				
MW (molecular weight; optimal: 100−600)	294.12	396.01	182.08	342.12
nHA (H-bond acceptors; optimal: 0−12)	7	8	6	11
nHD (H-bond donors; optimal: 0−7)	4	5	6	8
nRot (number of rotatable bonds; optimal: 0−11)	9	5	5	5
nRing (number of rings; optimal: 0−6)	1	2	0	2
maxring (atoms number in the biggest ring: 0−18)	6	6	0	6
nHet (number of heteroatoms; optimal: 1−15)	7	11	6	11
fChar (formal charge; optimal:−4 to 4)	0	0	0	0
nRig (number of rigid bonds; optimal: 0−30)	9	11	0	11
TPSA (topological polar surface area; optimal: 0−140)	118.72	128.84	121.38	189.53
log*S* (solubility; optimal; −4 to 0.5 log mol/L)	−1.228	−0.522	−0.0.01	0.087
log*P* (distribution coefficient *P*; optimal: 0−3)	−0.339	−1.012	−2.608	−3.206
log*D*7.4 (log*P* at physiological pH 7.4; optimal: 1−3)	−0.68	−0.772	−2.328	−2.56
Medicinal Chemistry				
QED (>0.67: excellent; ≤0.67: poor)	0.593	0.349	0.261	0.238
lipinski (MW ≤ 500; log*P* ≤ 5; nHA ≤ 10;nHD ≤ 5)	accepted	accepted	accepted	rejected
Pfizer (log*P* < 3; TPSA > 75)	accepted	accepted	accepted	accepted
GSK (MW ≤ 400; log*P* ≤ 4)	accepted	accepted	accepted	accepted
golden triangle (200 ≤ MW ≤ 50; −2 ≤ log*D* ≤ 5)	accepted	accepted	rejected	rejected
Absorption				
Caco-2 permeability (>−5.15: excellent; otherwise: poor)	poor	poor	poor	poor
Pgp-inhibitor (0−0.3: excellent; 0.7−1.0(++): poor)	no	no	no	no
Pgp-substrate (0−0.3: excellent; 0.7−1.0(++): poor)	no	medium	no	low
HIA (0−0.3: excellent; 0.7−1.0(++): poor)	high	low	low	low
Distribution				
PPB (≤90%: excellent; otherwise: poor)	low	low	low	low
VD (0.04−20: excellent; otherwise: poor)	high	high	high	high
BBB penetration (0−0.3: excellent; 0.7−1.0(++): poor)	medium	no	medium	medium
Fu (>20%: high Fu; 5−20%: medium Fu; <5% low Fu)	high	high	high	high
Metabolism [0: Nonsubstrate/Noninhibitor; 1: Substrate/Inhibitor; Probability of Being Substrate/Inhibitor (0−1)]				
CYP1A2 (inhibitor)	0.015	0.005	0.011	0.002
CYP2C19 (inhibitor)	0.055	0.008	0.005	0.003
CYP2C9 (inhibitor)	0.01	0.000	0.000	0.000
CYP2D6 (inhibitor)	0.024	0.001	0.000	0.000
CYP3A4 (inhibitor)	0.021	0.003	0.003	0.003
Excretion				
CL (clearance) ≥ 5: excellent; < 5: poor	high	poor	poor	poor
Toxicity				
hERG blockers (0−0.3: excellent; 0.7−1.0(++): poor)	no	no	no	no
H-HT (0−0.3: excellent; 0.7−1.0(++): poor)	no	no	no	no
AMES toxicity (0−0.3: excellent; 0.7−1.0(++): poor)	no	high toxicity	no	no
rat oral acute toxicity (0−0.3: excellent; 0.7−1.0(++): poor)	low toxicity	moderate toxicity	low toxicity	low toxicity
carcinogenicity (0−0.3: excellent; 0.7−1.0(++): poor)	no	moderate	no	no
Toxicophoric Rules				
acute toxicity rule	0 alerts	0 alerts	0 alerts	0 alerts
genotoxic carcinogenicity rule	0 alerts	4 alerts	0 alerts	0 alerts

interaction between methionine (Met S) and C(aro) results in an extra stabilization energy ranging from −1 to −1.5 kcal/mol, in contrast to a simple hydrophobic interaction. The methyl groups of leucine are the primary donors in protein−ligand interactions, with the two methyl groups of valine and other aliphatic amino acid residues, such as Ile and Ala. The acceptors in this case are ranked as in the case of C^{α}-H-Aro-π-interactions.[106] It is important to note that unfavorable bonds can greatly hinder the stability of the protein−ligand complex.[107]

3.3. Pharmacokinetic and Toxicity Properties (ADMET). The study utilized the ADMETlab 2.0 program to ascertain the ADMET characteristics of the substances employed. The results are provided in Table 7. As per the findings, aspartame and sucralose meet the physicochemical properties needed to be a drug. On the other hand, sorbitol and sucrose do not possess these properties. The data show that aspartame and sucralose have successfully passed Lipinski, Pfizer, GSK, and Golden Triangle drug similarity assessments. However, sorbitol and sucrose have poor QED values based on

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different drug-likeness-related properties, which led to their rejection observed by Lipinski and Golden Triangle (Table 7).

All of the sweeteners tested showed a low compatibility with Caco-2 permeability. Although none of the compounds were found to be P-glycoprotein (P-gp) inhibitors, sucrose and sorbitol have a high and moderate probability of being P-gp substrates. The P-gp enzymes function as efflux pumps, actively facilitating the outflow of medicines from cells and diminishing their intracellular concentrations. They safeguard the central nervous system (CNS) against the buildup of foreign substances and play a vital role in secretory processes.[108] Except for aspartame, the HIA values for all other examined sweeteners were low.

All of the examined sweeteners had a low plasma protein binding (PPB) rate, meaning they had a low chance of adsorbing onto the plasma proteins. This low PPB rate could contribute to the sweeteners having a high therapeutic index and therefore a low chance of toxicity. Additionally, the sweeteners showed high volumes of distribution (VD), as shown in Table 7. All sweeteners exhibited a significant proportion of unbound in plasma (Fu), indicating their propensity to traverse cellular membranes and efficiently reach their intended locations. Sucralose could not penetrate the BBB, while the other sweeteners had a moderate capacity, as shown in Table 7. The BBB is a protective barrier that hinders the entry of tiny and large molecules into the CNS. Nevertheless, this mechanism exclusively facilitates the transportation of molecules soluble in water and lipids. Additionally, it enables the targeted transportation of specific molecules and medications, particularly those that are substrates of active transporters like glucose and P-gp transporters.[109] According to Md Idris et al.,[110] the hydrophilic nature of the compounds affects their distribution properties, such as HIA, PPB, VD, Fu, and BBB.

The human cytochrome family, which consists of 57 types of enzymes, is responsible for metabolizing around two-thirds of all known drugs in humans. Among these enzymes, five isozymes (1A2, 3A4, 2C9, 2C19, and 2D6) carry out 80% of drug metabolism. As per the data in Table 7, all sweeteners examined were noninhibitors of these enzymes. It is of utmost importance to refrain from using chemicals that impede the activity of CYP450 enzymes, as this could potentially give rise to significant drug−drug interactions and could lead to toxicity or pharmacokinetic augmentation.[111]

Sweeteners other than aspartame have low clearance rates, which means they accumulate in the body for more extended periods. This accumulation affects the bioavailability and half-life of drugs, directly affecting the dosage and frequency of the medication. While low clearance rates can be beneficial as they require less frequent dosing, they can also increase a compound's toxicity if it is concentration-dependent.[109] A human ether-a-go-go-related gene (hERG) encodes a voltage-gated potassium channel that controls the regulation of the heart's action potential and resting potential. Blocking hERG can lead to sudden death.[112] However, the findings of Table 7 stated that sweeteners do not block the hERG K+ channel and are generally safe, except for sucralose, which has shown high toxicity in the Ames test for mutagenicity, moderate carcinogenicity, and rat oral acute toxicity. Testing acute toxicity in mammals is crucial to evaluate drug safety.[24] The ADMET results suggest that the sweeteners investigated have promising pharmacokinetic properties with a minimum toxicity. However, the adverse properties of sweeteners in conjunction with in vivo and in silico results, especially for aspartame, showed the potential effect against proteins and hormones studies, indicating the need for further toxicological studies to ensure their safety (Table 7). Such necessity agreed with the study of Anand et al.[113] who showed various pathological complications arise in many organs, including the brain, heart, liver, and lungs, due to the excess consumption of aspartame. In addition, methanol, which is a byproduct of aspartame hydrolysis, has diverse toxic effects, such as its direct link to AD pathology.[113]

4. CONCLUSIONS

The current study evaluated the effects of artificial (aspartame and sucralose) and natural sweeteners (sorbitol and sucrose) on male rats after 12 weeks of administration. Sweeteners increase body weight and food intake, especially aspartame, which had the highest impact. All sweeteners reduced hemoglobin levels and increased the levels of TSH, PTH, and prostaglandin E2 hormones. Aspartame caused the highest decline in steroid hormones and a significant increase in anti- and proinflammatory cytokines. An in silico study showed that sweeteners or ligands, particularly aspartame, bind more strongly to target proteins related to hormones or cytokines such as thyroid, leptin, and prostaglandin E2. This binding confirms the mechanism of action that leads to hormonal and cytokine changes observed during the in vivo study. Additionally, based on drug-likeness-related properties computed by ADMET, aspartame, and sucralose successfully passed Lipinski, Pfizer, GSK, and Golden Triangle drug similarity assessments, in contrast to sucrose and sorbitol.

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DMM: Conceptualization, investigation, formal analysis, methodology, interpretation of biological data, statistical analysis, data curation, and writing—software review and editing. **MAA, MMG, AA, RHA:** Revision and editing. **MMG, AA, RHA:** Funding acquisition. **AF:** Conceptualization, project administration, investigation, writing—original draft, method-

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Notes

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■ REFERENCES

(1) Suez, J.; Korem, T.; Zeevi, D.; Zilberman-Schapira, G.; Thaiss, C. A.; Maza, O.; Israeli, D.; Zmora, N.; Gilad, S.; Weinberger, A.; et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* **2014**, *514* (7521), 181−186.

(2) Jain, T.; Grover, K. Sweeteners in human nutrition. *Int. J. Med. Health Sci. Res.* **2015**, *5* (5), 439−451.

(3) Rahiman, F.; Pool, E. J. The in vitro effects of artificial and natural sweeteners on the immune system using whole blood culture assays. *J. Immunoassay Immunochem.* **2014**, *35* (1), 26−36.

(4) Zygler, A.; Wasik, A.; Namieśnik, J. Analytical methodologies for determination of artificial sweeteners in foodstuffs. *Trac. Trends Anal. Chem.* **2009**, *28* (9), 1082−1102.

(5) Del Pozo, S.; Gómez-Martínez, S.; Díaz, L. E.; Nova, E.; Urrialde, R.; Marcos, A. Potential effects of Sucralose and saccharin on gut microbiota: a review. *Nutrients.* **2022**, *14* (8), 1682.

(6) Bhoumik, S.; Kumar, R.; Srivastava, P.; Singh, A.; Siddiqui, Z.; Rizvi, S. I. Investigation of the toxic effects of aspartame as an artificial sweetener in food: effect on redox and inflammatory biomarkers in rat. *J. Food Hyg. Saf.* **2023**, *9* (2), 99−107.

(7) Mongkhon, J. M.; Thach, M.; Shi, Q.; Fernandes, J. C.; Fahmi, H.; Benderdour, M. Sorbitol-modified hyaluronic acid reduces oxidative stress, apoptosis and mediators of inflammation and catabolism in human osteoarthritic chondrocytes. *Inflammation Res.* **2014**, *63*, 691−701.

(8) Rosales-Gómez, C. A.; Martínez-Carrillo, B. E.; Reséndiz-Albor, A. A.; Ramírez-Durán, N.; Valdés-Ramos, R.; Mondragón-Velásquez, T.; Escoto-Herrera, J. A. Chronic consumption of sweeteners and its effect on glycaemia, cytokines, hormones, and lymphocytes of GALT in CD1 mice. *BioMed Res. Int.* **2018**, *2018*, 1345282.

(9) Zhang, R.; Noronha, J. C.; Khan, T. A.; McGlynn, N.; Back, S.; Grant, S. M.; Kendall, C. W. C.; Sievenpiper, J. L. The effect of non-nutritive sweetened beverages on postprandial glycemic and endocrine responses: a systematic review and network meta-analysis. *Nutrients.* **2023**, *15* (4), 1050.

(10) Naik, A. Q.; Zafar, T.; Shrivastava, V. K. The impact of non-caloric artificial sweetener aspartame on female reproductive system in mice model. *Reprod. Biol. Endocrinol.* **2023**, *21* (1), 73.

(11) Anbara, H.; Sheibani, M. T.; Razi, M. Long-term effect of aspartame on male reproductive system: Evidence for testicular histomorphometrics, Hsp70−2 protein expression and biochemical status. *Int J Fertil Steril.* **2020**, *14* (2), 91.

(12) Abbas, D. M.; Kadhim, R. A.; Abed, S. N. The Effect of Acesulfame k and Aspartame as Sweetener Materials in Food Products on Body Parameters of Rats. *Journal of Population Therapeutics and Clinical Pharmacology.* **2023**, *30* (5), 607−614.

(13) Maty, H. N. Impact of sorbitol and l-carnitine on stimulating thyroid hormone, triiodothyronine and adenosine triphosphate level in broilers. *Iraqi J. Vet. Sci.* **2023**, *37* (3), 589−590.

(14) AOAC. *Official Methods of Analysis of AOAC International*, 15th ed.; AOAC International: Gaithersburg, MD, USA, 2009.

(15) Sabry, B. A.; Badr, A. N.; Ahmed, K. A.; Desoukey, M. A.; Mohammed, D. M. Utilizing lemon peel extract and its nano-emulsion to control aflatoxin toxicity in rats. *Food Biosci.* **2022**, *50*, 101998.

(16) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* **1993**, *123*, 1939−1951.

(17) Azeez, O. H. Evaluation of Some Male and Female Rats' Reproductive Hormones Following Administration of Aspartame with or Without Vitamin C or E. *Vet. Med.* **2021**, *45* (2), 14−20.

(18) Chattopadhyay, S.; Raychaudhuri, U.; Chakraborty, R. Artificial sweeteners-a review. *J. Food Sci. Technol.* **2014**, *51*, 611−621.

(19) Abukhomra, A. S.; Al-Awady, H. G.; Al-Charak, A. H. Effect of Different Doses of Sorbitol on Hematological Parameters and Blood Glucose of Rats. *J. Pharm. Negat. Results* **2022**, *13*, 1765−1771.

(20) Carrasco Cabrera, L.; Medina Pastor, P.; European Food Safety Authority EFSA. The 2020 European Union report on pesticide residues in food. *EFSA J.* **2022**, *20* (3), No. e07215.

(21) Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *J. Cheminf.* **2012**, *4* (1), 17.

(22) Liu, Y.; Grimm, M.; Dai, W. T.; Hou, M. C.; Xiao, Z. X.; Cao, Y. CB-Dock: A web server for cavity detection-guided protein-ligand blind docking. *Acta Pharmacol. Sin.* **2020**, *41* (1), 138−144.

(23) Mohammed, D. M.; Abdelgawad, M. A.; Ghoneim, M. M.; El-Sherbiny, M.; Mahdi, W. A.; Alshehri, S.; Ebrahim, H. A.; Farouk, A. Effect of nano-encapsulated food flavorings on streptozotocin-induced diabetic rats. *Food Funct.* **2023**, *14* (19), 8814−8828.

(24) Xiong, G.; Wu, Z.; Yi, J.; Fu, L.; Yang, Z.; Hsieh, C.; Yin, M.; Zeng, X.; Wu, C.; Lu, A.; et al. ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Res.* **2021**, *49* (W1), W5−W14.

(25) *Sweeteners and Sugar Alternatives in Food Technology*; O'Donnell, K., Kearsley, M. W., Eds.; John Wiley & Sons 2012.

(26) Mbambo, N. P.; Dlamini, S. N.; Chukwuma, C. I.; Islam, M. S. Comparative effects of commonly used commercially available non-nutritive sweeteners on diabetes-related parameters in non-diabetic rats. *J. Food Biochem.* **2020**, *44* (11), No. e13453.

(27) Prokic, M. D.; Paunovic, M. G.; Matic, M. M.; Djordjevic, N. Z.; Ognjanovic, B. I.; Stajn, A. S.; Saicic, Z. S. Effect of aspartame on biochemical and oxidative stress parameters in rat blood. *Arch. Biol. Sci.* **2015**, *67* (2), 535−545.

(28) Brown, R. J.; De Banate, M. A.; Rother, K. I. Artificial sweeteners: a systematic review of metabolic effects in youth. *Int. J. Pediatr. Obes.* **2010**, *5* (4), 305−312.

(29) Yang, Q. Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings: Neuroscience 2010. *Yale J. Biol. Med.* **2010**, *83* (2), 101.

(30) de Matos Feijó, M.; Ballard, C. R.; Foletto, K. C.; Batista, B. A. M.; Neves, A. M.; Ribeiro, M. F. M.; Bertoluci, M. C. Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels. *Appetite.* **2013**, *60*, 203−207.

(31) Choudhary, A. K.; Devi, R. S. Serum biochemical responses under oxidative stress of aspartame in wistar albino rats. *Asian Pac. J. Trop. Dis.* **2014**, *4*, S403−S410.

(32) Bian, X.; Chi, L.; Gao, B.; Tu, P.; Ru, H.; Lu, K. The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *PloS one.* **2017**, *12* (6), No. e0178426.

(33) Gul, S. S.; Hamilton, A. R. L.; Munoz, A. R.; Phupitakphol, T.; Liu, W.; Hyoju, S. K.; Economopoulos, K. P.; Morrison, S.; Hu, D.; Zhang, W.; et al. Inhibition of the gut enzyme intestinal alkaline phosphatase may explain how aspartame promotes glucose intolerance and obesity in mice. *Appl. Physiol., Nutr., Metab.* **2017**, *42* (1), 77−83.

(34) Nagao, T.; Hirokawa, M. Diagnosis and treatment of macrocytic anemias in adults. *Journal of general and family medicine.* **2017**, *18* (5), 200−204.

https://doi.org/10.1021/acsomega.4c01250
ACS Omega 2024, 9, 30364−30380

(35) Hariz, A.; Bhattacharya, P. T. *Megaloblastic Anemia*. [Updated 2021 Oct 11]. StatPearls [Internet]; StatPearls Publishing: Treasure Island (FL), 2022.
(36) Moore, C. A.; Adil, A. *Macrocytic Anemia*. In StatPearls [Internet]; StatPearls Publishing,2022.
(37) Manna, P.; Jain, S. K. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metab. Syndr. Relat. Disord.* **2015**, *13* (10), 423−444.
(38) Chukwuma, C. I.; Islam, S. Xylitol improves anti-oxidative defense system in serum, liver, heart, kidney and pancreas of normal and type 2 diabetes model of rats. *Acta Pol. Pharm.* **2017**, *74* (3), 817.
(39) Adkison, K.; Wolstenholme, A.; Lou, Y.; Zhang, Z.; Eld, A.; Perger, T.; Vangerow, H.; Hayward, K.; Shaefer, M.; McCoig, C. Effect of sorbitol on the pharmacokinetic profile of lamivudine oral solution in adults: an open-label, randomized study. *Clin. Pharmacol. Ther.* **2018**, *103* (3), 402−408.
(40) Liauw, S.; Saibil, F. Sorbitol: Often forgotten cause of osmotic diarrhea. *Can Fam Physician.* **2019**, *65* (8), 557−558.
(41) Nakagawa, Y.; Nagasawa, M.; Yamada, S.; Hara, A.; Mogami, H.; Nikolaev, V. O.; Lohse, M. J.; Shigemura, N.; Ninomiya, Y.; Kojima, I. Sweet Taste Receptor Expressed in Pancreatic β-Cells Activates the Calcium and Cyclic AMP Signaling Systems and Stimulates Insulin Secretion. *PloS one.* **2009**, *4* (4), No. e5106.
(42) Pang, M. D.; Goossens, G. H.; Blaak, E. E. The impact of artificial sweeteners on body weight control and glucose homeostasis. *Front. Nutr.* **2021**, *7*, 333.
(43) Gupta, S.; Kalra, S.; Bharihoke, V.; Dhurandhar, D. Sucralose induced pancreatic toxicity in albino rats: Histomorphological evidence. *J. Morphol. Sci.* **2014**, *31* (02), 123−127.
(44) Lertrit, A.; Srimachai, S.; Saetung, S.; Chanprasertyothin, S.; Chailurkit, L. O.; Areevut, C.; Katekao, P.; Ongphiphadhanakul, B.; Sriphrapradang, C. Effects of sucralose on insulin and glucagon-like peptide-1 secretion in healthy subjects: a randomized, double-blind, placebo-controlled trial. *Nutrition.* **2018**, *55*−*56*, 125−130.
(45) Alshafei, M. M.; Mohammed, D.; Hanafi, E. M.; Kassem, S. S.; El-Messery, T. M.; Korany, R.; Elsaid, M. M. Red Beet Peel-Loaded Liposome's Impact on Metabolic Syndrome Rats and Its Use as Fortifier in Yoghurt Powder. *Egypt. J. Chem.* **2023**, *66* (2), 517−533.
(46) Lutz, T. A. Control of energy homeostasis by amylin. *Cell. Mol. Life Sci.* **2012**, *69*, 1947−1965.
(47) Aston-Mourney, K.; Hull, R. L.; Zraika, S.; Udayasankar, J.; Subramanian, S. L.; Kahn, S. E. Exendin-4 increases islet amyloid deposition but offsets the resultant beta cell toxicity in human islet amyloid polypeptide transgenic mouse islets. *Diabetologia* **2011**, *54*, 1756−1765.
(48) St-Pierre, P.; Pilon, G.; Dumais, V.; Dion, C.; Dubois, M. J.; Dubé, P.; Desjardins, Y.; Marette, A. Comparative analysis of maple syrup to other natural sweeteners and evaluation of their metabolic responses in healthy rats. *J. Funct.Foods* **2014**, *11*, 460−471.
(49) Wu, T.; Bound, M. J.; Standfield, S. D.; Bellon, M.; Young, R. L.; Jones, K. L.; Horowitz, M.; Rayner, C. K. Artificial sweeteners have no effect on gastric emptying, glucagon-like peptide-1, or glycemia after oral glucose in healthy humans. *Diabetes care.* **2013**, *36* (12), e202−e203.
(50) Baggio, L. L.; Drucker, D. J. Biology of incretins: GLP-1 and GIP. *Gastroenterology* **2007**, *132* (6), 2131−2157.
(51) Saada, H. N.; Mekky, N. H.; Eldawy, H. A.; Abdelaal, A. F. Biological effect of sucralose in diabetic rats. *Food Nutr. Sci.* **2013**, *04* (07), 82−89.
(52) Parker, H. E.; Reimann, F.; Gribble, F. M. Molecular mechanisms underlying nutrient-stimulated incretin secretion. *Expet Rev. Mol. Med.* **2010**, *12*, No. e1.
(53) Steinert, R. E.; Frey, F.; Töpfer, A.; Drewe, J.; Beglinger, C. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. *Br. J. Nutr.* **2011**, *105* (9), 1320−1328.
(54) Alshafei, M. M.; Mabrouk, A. M.; Hanafi, E. M.; Ramadan, M. M.; Korany, R. M.; Kassem, S. S.; Mohammed, D. M. Prophylactic supplementation of microencapsulated Boswellia serrata and probiotic bacteria in metabolic syndrome rats. *Food Biosci.* **2023**, *51*, 102325.
(55) Fernstrom, J. D.; Munger, S. D.; Sclafani, A.; de Araujo, I. E.; Roberts, A.; Molinary, S. Mechanisms for sweetness. *J. Nutr.* **2012**, *142* (6), 1134S−1141S.
(56) Laffitte, A.; Neiers, F.; Briand, L. Functional roles of the sweet taste receptor in oral and extraoral tissues. *Curr. Opin. Clin. Nutr. Metab. Care* **2014**, *17* (4), 379−385.
(57) Kojima, I.; Nakagawa, Y. The Role of the Sweet Taste Receptor in Enteroendocrine Cells and Pancreatic β-Cells. *Diabetes Metab J.* **2011**, *35* (5), 451−457.
(58) Cui, M.; Jiang, P.; Maillet, E.; Max, M.; Margolskee, R. F.; Osman, R. The heterodimeric sweet taste receptor has multiple potential ligand binding sites. *Curr. Pharmaceut. Des.* **2006**, *12* (35), 4591−4600.
(59) Liauchonak, I.; Qorri, B.; Dawoud, F.; Riat, Y.; Szewczuk, M. R. Non-nutritive sweeteners and their implications on the development of metabolic syndrome. *Nutrients.* **2019**, *11* (3), 644.
(60) Han, P.; Bagenna, B.; Fu, M. The sweet taste signalling pathways in the oral cavity and the gastrointestinal tract affect human appetite and food intake: A review. *Int. J. Food Sci. Nutr.* **2019**, *70* (2), 125−135.
(61) Helal, E. G.; Al-Shamrani, A.; Abdelaziz, M. A.; El-Gamal, M. S. Comparison between the effect of sucralose and sodium saccharin on some physiological parameters in male albino rats. *Egypt. J. Hosp. Med.* **2019**, *74* (7), 1552−1558.
(62) Bringhurst, F. R.; Demay, M. B.; Kronenberg, H. M. Hormones and disorders of mineral metabolism. *Williams Textbook of Endocrinology*; Elsevier, 2011; pp 1237−1304.
(63) Volkov, E. E.; Goloshchapov, A. P.; Mustafin, R. N.; Nostaeva, S. E. Factor analysis of clinical and biochemical parameters of bone remodeling changes associated with leading VDR polymorphisms in patients with aseptic necrosis of the femoral head. *Genius of Orthopedics.* **2023**, *29* (1), 57−63.
(64) Kawahara, K.; Hohjoh, H.; Inazumi, T.; Tsuchiya, S.; Sugimoto, Y. Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2015**, *1851* (4), 414−421.
(65) Pałkowska-Goździk, E.; Bigos, A.; Rosołowska-Huszcz, D. Type of sweet flavour carrier affects thyroid axis activity in male rats. *Eur. J. Nutr.* **2018**, *57*, 773−782.
(66) Sachmechi, I.; Khalid, A.; Awan, S. I.; Malik, Z. R.; Sharifzadeh, M. Autoimmune thyroiditis with hypothyroidism induced by sugar substitutes. *Cureus.* **2018**, *10* (9), No. e3268.
(67) Oliynyk, O. The influence of sucralose on thyroid gland function. *Health Problems of Civilization.* **2021**, *15* (1), 12−16.
(68) Celec, P.; Ostatníková, D.; Hodosy, J. On the effects of testosterone on brain behavioral functions. *Front. Neurosci.* **2015**, *9*, 12.
(69) Ohlander, S. J.; Lindgren, M. C.; Lipshultz, L. I. Testosterone and male infertility. *Urol. Clin.* **2016**, *43* (2), 195−202.
(70) Taraborrelli, S. Physiology, production and action of progesterone. *Acta Obstet. Gynecol. Scand.* **2015**, *94*, 8−16.
(71) Sundström-Poromaa, I.; Comasco, E.; Sumner, R.; Luders, E. Progesterone-Friend or foe? *Front. Neuroendocrinol.* **2020**, *59*, 100856.
(72) Ohlsson, C.; Langenskiöld, M.; Smidfelt, K.; Poutanen, M.; Ryberg, H.; Norlén, A. K.; Nordanstig, J.; Bergström, G.; Tivesten, Å. Low progesterone and low estradiol levels associate with abdominal aortic aneurysms in men. *J. Clin. Endocrinol. Metab.* **2022**, *107* (4), No. e1413.
(73) Ramasamy, R.; Schulster, M.; Bernie, A. The role of estradiol in male reproductive function. *Asian J. Androl.* **2016**, *18* (3), 435.
(74) Hanafi, E. M.; Korany, R.; Tawfeek, M.; Hozyen, H. F.; El-Nattat, W. S.; Ramadan, M. M.; Kassem, S.; Alshafei, M. M.; Mohammed, D. Effect of Frankincense (Boswellia Carterii) on Animal Reproductive Performance. *Egypt. J. Chem.* **2023**, *66* (2), 213.
(75) Yin, K. J.; Xie, D. Y.; Zhao, L.; Fan, G.; Ren, J. N.; Zhang, L. L.; Pan, S. Y. Effects of different sweeteners on behavior and

https://doi.org/10.1021/acsomega.4c01250
ACS Omega 2024, 9, 30364−30380

neurotransmitters release in mice. *J. Food Sci. Technol.* **2020**, *57*, 113−121.
(76) Dalal, R.; Grujic, D. *Epinephrine*. In StatPearls [Internet]; StatPearls Publishing, 2023.
(77) Jordan, J.; Grassi, G. The norepinephrine transporter deserves more attention. *J. Hypertens.* **2018**, *36* (7), 1472−1474.
(78) Mruk, D. D.; Cheng, C. Y. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr. Rev.* **2004**, *25* (5), 747−806.
(79) Salama, M.; Mohammed, D. M.; Fahmy, K.; Al-Senosy, N. K.; Ebeed, N. M.; Farouk, A. Evaluation of the cytotoxicity and genotoxicity potential of synthetic diacetyl food flavoring in silico, in vivo, and in vitro. *Food Chem. Toxicol.* **2023**, *178*, 113923.
(80) Calder, P. C.; Ahluwalia, N.; Albers, R.; Bosco, N.; Bourdet-Sicard, R.; Haller, D.; Holgate, S.; Jönsson, L.; Latulippe, M.; Marcos, A.; et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. *Br. J. Nutr.* **2013**, *109* (S1), S1−S34.
(81) Soliman, T. N.; Mohammed, D. M.; El-Messery, T. M.; Elaaser, M.; Zaky, A. A.; Eun, J. B.; Shim, J. H.; El-Said, M. M. Microencapsulation of plant phenolic extracts using complex coacervation incorporated in ultrafiltered cheese against AlCl3-induced neuroinflammation in rats. *Front. Nutr.* **2022**, *9*, 929977.
(82) Compare, D.; Coccoli, P.; Rocco, A.; Nardone, O. M.; De Maria, S.; Cartenì, M.; Nardone, G. Gut-liver axis: the impact of gut microbiota on non-alcoholic fatty liver disease. *Nutr. Metabol. Cardiovasc. Dis.* **2012**, *22* (6), 471−476.
(83) Czaja, A. J. Hepatic inflammation and progressive liver fibrosis in chronic liver disease. *World journal of gastroenterology: WJG.* **2014**, *20* (10), 2515.
(84) Mostafa Mohammed, D.; El-Messery, T. M.; Baranenko, D. A.; Hashim, M. A.; Tyutkov, N.; Marrez, D. A.; Elmessery, W. M.; El-Said, M. M. Effect of Spirulina maxima microcapsules to mitigate testicular toxicity induced by cadmium in rats: Optimization of in vitro release behavior in the milk beverage. *J. Funct.Foods* **2024**, *112*, 105938.
(85) Damián-Medina, K.; Salinas-Moreno, Y.; Milenkovic, D.; Figueroa-Yáñez, L.; Marino-Marmolejo, E.; Higuera-Ciapara, I.; Vallejo-Cardona, A.; Lugo-Cervantes, E. In silico analysis of antidiabetic potential of phenolic compounds from blue corn (Zea mays L.) and black bean (Phaseolus vulgaris L.). *Heliyon.* **2020**, *6* (3), No. e03632.
(86) Ahn, C. H.; Oh, T. J.; Min, S. H.; Cho, Y. M. Incretin and Pancreatic β-Cell Function in Patients with Type 2 Diabetes. *Endocrinol. Metab.* **2023**, *38* (1), 1−9.
(87) Munikumar, M.; Krishna, V. S.; Reddy, V. S.; Rajeswari, B.; Sriram, D.; Rao, M. V. In silico design of small peptides antagonist against leptin receptor for the treatment of obesity and its associated immune-mediated diseases. *J. Mol. Graphics Modell.* **2018**, *82*, 20−36.
(88) Alkahtane, A. A.; Alghamdi, H. A.; Almutairi, B.; Khan, M. M.; Hasnain, M. S.; Abdel-Daim, M. M.; Alghamdi, W. M.; Alkahtani, S. Inhibition of human amylin aggregation by Flavonoid Chrysin: An in-silico and in-vitro approach. *Int. J. Med. Sci.* **2021**, *18* (1), 199−206.
(89) Fang, M.; Zhang, Q.; Wang, X.; Su, K.; Guan, P.; Hu, X. Inhibition Mechanisms of (−)-Epigallocatechin-3-gallate and Genistein on Amyloid-beta 42 Peptide of Alzheimer's Disease via Molecular Simulations. *ACS omega.* **2022**, *7* (23), 19665−19675.
(90) Bhogireddy, N.; Veeramachaneni, G. K.; Ambatipudi, N. V. K.; Mathi, P.; Ippaguntla, J.; Ganta, U. R.; Adusumalli, S.; Bokka, V. Inferences from the ADMET analysis of predicted inhibitors to follicle stimulating hormone in the context of infertility. *Bioinformation.* **2013**, *9* (15), 788−791.
(91) Mingione, A.; Maruca, K.; Chiappori, F.; Pivari, F.; Brasacchio, C.; Quirino, T.; Merelli, I.; Soldati, L.; Bonfanti, P.; Mora, S. High parathyroid hormone concentration in tenofovir-treated patients are due to inhibition of calcium-sensing receptor activity. *Biomed. Pharmacother.* **2018**, *97*, 969−974.
(92) Shaker, M. E.; Goma, H. A.; Alsalahat, I.; Elkanzi, N. A.; Azouz, A. A.; Abdel-Bakky, M. S.; Ghoneim, M. M.; Hazem, S. H.; El-Mesery, M. E.; Farouk, A.; et al. Design and construction of novel pyridine-pyrimidine hybrids as selective COX-2 suppressors: anti-inflammatory potential, ulcerogenic profile, molecular modeling and ADME/Tox studies. *J. Biomol. Struct. Dyn.* **2023**, 1−14.
(93) Tai, H. H. Prostaglandin catabolic enzymes as tumor suppressors. *Cancer Metastasis Rev.* **2011**, *30*, 409−417.
(94) Lesmana, R.; Shidqi, F. R.; Goenawan, H.; Setiawan, I.; Gondokesumo, M. E.; Suhud, F.; Wathoni, N. The potential interaction of ethionamide-thyroid hormone receptor induces hypothyroidism. *Phcog. J.* **2021**, *13* (5), 1174−1179.
(95) Kar, A.; Panda, S.; Singh, M.; Biswas, S. Regulation of PTU-induced hypothyroidism in rats by caffeic acid primarily by activating thyrotropin receptors and by inhibiting oxidative stress. *Phytomed. Plus* **2022**, *2* (3), 100298.
(96) Gomes, A. R. Q.; Cruz, J. N.; Castro, A. L. G.; Cordovil Brigido, H. P.; Varela, E. L. P.; Vale, V. V.; Carneiro, L. A.; Ferreira, G. G.; Percario, S.; Dolabela, M. F. Participation of oxidative stress in the activity of compounds isolated from Eleutherine plicata herb. *Molecules* **2023**, *28* (14), 5557.
(97) Li, J.; Tian, F.; Tang, Y.; Shi, L.; Wang, S.; Hu, Z.; Zhu, Y.; Wang, Y.; Li, H.; Ge, R. s.; et al. Inhibition of human and rat placental 3β-hydroxysteroid dehydrogenase/Δ5,4-isomerase activities by insecticides and fungicides: Mode action by docking analysis. *Chem.-Biol. Interact.* **2023**, *369*, 110292.
(98) Mori Sequeiros García, M.; Acquier, A.; Suarez, G.; Gomez, N. V.; Gorostizaga, A.; Mendez, C. F.; Paz, C. Cisplatin inhibits testosterone synthesis by a mechanism that includes the action of reactive oxygen species (ROS) at the level of P450scc. *Chem.-Biol. Interact.* **2012**, *199* (3), 185−191.
(99) DeVore, N. M.; Scott, E. E. Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001. *Nature* **2012**, *482* (7383), 116−119.
(100) Prafulla, S.; Lata, P. Molecular Docking Simulation Study of Novel Flavones and Flavonoids as Aromatase Inhibitors. *Der Pharma Chem.* **2018**, *10* (12), 68.
(101) Shah, U.; Patel, S.; Patel, M.; Upadhayay, J. Molecular docking and in silico admet study reveals flavonoids as a potential inhibitor of aromatase. *Lett. Drug Des. Discovery* **2017**, *14* (11), 1267−1276.
(102) Ferreira de Freitas, R.; Schapira, M. A systematic analysis of atomic protein-ligand interactions in the PDB. *Medchemcomm.* **2017**, *8* (10), 1970−1981.
(103) Tsuzuki, S.; Honda, K.; Uchimaru, T.; Mikami, M.; Tanabe, K. Origin of attraction and directionality of the π/π interaction: model chemistry calculations of benzene dimer interaction. *J. Am. Chem. Soc.* **2002**, *124* (1), 104−112.
(104) Arthur, D. E.; Akoji, J. N.; Sahnoun, R.; Okafor, G. C.; Abdullahi, K. L.; Abdullahi, S. A.; Mgbemena, C. A theoretical insight in interactions of some chemical compounds as mTOR inhibitors. *Bull Natl Res Cents* **2021**, *45*, 67.
(105) Valley, C. C.; Cembran, A.; Perlmutter, J. D.; Lewis, A. K.; Labello, N. P.; Gao, J.; Sachs, J. N. The methionine-aromatic motif plays a unique role in stabilizing protein structure. *J. Biol. Chem.* **2012**, *287* (42), 34979−34991.
(106) Brandl, M.; Weiss, M. S.; Jabs, A.; Sühnel, J.; Hilgenfeld, R. C-h···π-interactions in proteins. *J. Mol. Biol.* **2001**, *307* (1), 357−377.
(107) Prabhakaran, P.; Hebbani, A. V.; Menon, S. V.; Paital, B.; Murmu, S.; Kumar, S.; Singh, M. K.; Sahoo, D. K.; Desai, P. P. D. Insilico generation of novel ligands for the inhibition of SARS-CoV-2 main protease (3CLpro) using deep learning. *Front. Microbiol.* **2023**, *14*, 1194794.
(108) Obakiro, S. B.; Kiprop, A.; K'owino, I.; Andima, M.; Owor, R. O.; Chacha, R.; Kigondu, E. Phytochemical, Cytotoxicity, and Antimycobacterial Activity Evaluation of Extracts and Compounds from the Stem Bark of Albizia coriaria Welw ex. Oliver. *Evidence-Based Complementary Altern. Med.* **2022**, *2022*, 1−20.
(109) Krüger, A.; Gonçalves Maltarollo, V.; Wrenger, C.; Kronenberger, T. ADME profiling in drug discovery and a new

https://doi.org/10.1021/acsomega.4c01250
ACS Omega 2024, 9, 30364−30380

path paved on silica. *Drug Discovery and Development-New Advances*; IntechOpen, 2019; pp 1−30.
(110) Md Idris, M. H.; Mohd Amin, S. N.; Mohd Amin, S. N.; Wibowo, A.; Zakaria, Z. A.; Shaameri, Z.; Hamzah, A. S.; Selvaraj, M.; Teh, L. K.; Salleh, M. Z. Discovery of polymethoxyflavones as potential cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX) and phosphodiesterase 4B (PDE4B) inhibitors. *J. Recept. Signal Transduction* **2022**, *42* (4), 325−337.
(111) Durán-Iturbide, N. A.; Díaz-Eufracio, B. I.; Medina-Franco, J. L. In silico ADME/Tox profiling of natural products: A focus on BIOFACQUIM. *ACS omega.* **2020**, *5* (26), 16076−16084.
(112) Adem, Ş.; Yırtıcı, U.···; Aydın, M.; Rawat, R.; Eyüpoğlu, V. Natural flavonoids as promising 6-phosphogluconate dehydrogenase inhibitor candidates: In silico and in vitro assessments. *Archiv der Pharmazie.* **2024**, *357* (1), 2300326.
(113) Anand, B. G.; Prajapati, K. P.; Dubey, K.; Ahamad, N.; Shekhawat, D. S.; Rath, P. C.; Joseph, G. K.; Kar, K. Self-Assembly of Artificial Sweetener Aspartame Yields Amyloid-like Cytotoxic Nanostructures. *ACS Nano* **2019**, *13* (5), 6033−6049.

https://doi.org/10.1021/acsomega.4c01250
ACS Omega 2024, 9, 30364−30380