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Sex-Dependent Effects of Sodium Saccharin and Antioxidant Vitamins on Reproductive Hormones in Wistar Rats at Adi Levels

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Abstract

Non-nutritive sweeteners like sodium saccharin face growing scrutiny for their potential endocrine-disrupting effects on reproductive health. This study investigates sodium saccharin's impact on reproductive hormones in male and female Wistar rats, and the modulatory roles of antioxidants vitamins C and E. Eighty adult rats were divided into four groups: control (Group 1); sodium saccharin alone at 5 mg/kg body weight (Group 2); sodium saccharin plus vitamin C at 150 mg/kg (Group 3); and sodium saccharin plus vitamin E at 100 mg/kg (Group 4). Treatments were administered orally for 90 days, followed by serum assays for estradiol, follicle-stimulating hormone, luteinizing hormone, and testosterone. In males, sodium saccharin elevated estradiol by 52% but suppressed follicle-stimulating hormone by 22% versus controls. Vitamin C co-administration further increased estradiol by 22% (compared to sodium saccharin alone) and testosterone by 58% (compared to both control and sodium saccharin groups). Vitamin E countered this by boosting follicle-stimulating hormone and testosterone by 32% and 23%, respectively, relative to controls and sodium saccharin groups. In females, sodium saccharin raised follicle-stimulating hormone 42%, estradiol 42%, and luteinizing hormone 29%, while reducing testosterone 38% versus controls. Vitamin C with sodium saccharin amplified follicle-stimulating hormone by 19% but decreased testosterone 23% (compared to controls). Vitamin E showed superior restoration of gonadotropin balance. In conclusion, sodium saccharin disrupts the reproductive endocrine axis in sex-specific ways, increasing estradiol levels in males and elevated gonadotropin levels in females. Vitamin E effectively improved gonadotropins, while vitamin C showed a greater effect on testosterone levels, highlighting differential antioxidant protective mechanisms.

Keywords: Sodium saccharin, Antioxidant vitamins, Endocrine disruption, Rats; Reproductive hormones.

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Introduction

Over the past decades, the use of non-nutritive sweeteners (NAS), such as sodium saccharin (Sod.S.), has substantially increased due to their use as sugar substitutes in various foods and drinks intended to prevent increased sugar consumption and consequently improve glucose metabolism (1,2). These sweeteners are particularly beneficial for the health of diabetic and overweight/obese people since they provide a means of preventing possible complications caused by metabolic diseases, particularly in health-conscious populations (2). Besides medicinal approaches, low-sugar diets with specific carbohydrate portions remain the easiest way to improve overall health (3). Of all the non-nutritive sweeteners used today, Sod.S. remains the most used sweetener in the world due to its sweet properties and the long history of its use in making food products (4,5). Saccharin is 300 times sweeter than sugar and used extensively as a tabletop sweetener, in soft drinks, confectionery products, and pharmaceutical formulations (6,7). It is resistant to metabolic degradation and, therefore, can pass through the gastrointestinal tract largely unaltered, which makes it especially attractive to diabetes or body-weight control patients (8–10). Various regulatory agencies like the Food and Drug Administration in the USA and the Joint FAO/WHO Expert Committee on Food Additives have recognized the safe use of Sod.S. when ingested within the permissible daily allowance of the acceptable daily intake (ADI) level, which has been cited as 5 mg/kg body weight (BW) (11,12). However, in recent years, there has been an increase in interest in the biological activity of chronic saccharin ingestion in a manner that is not evidenced as toxicity. This contrasts with obvious toxicity or carcinogenesis, as endocrine effects can be present in low doses during chronic exposure modes in a manner that fails to be recognized as toxicity (13,14). Sod.S. may be recognized as "safe" in food products, but concerns over its potential to cause long-term health risks include those cited for some artificial sweetener-related carcinogenesis in animal studies (15,16).

Artificial sweeteners may influence reproductive hormones through mechanisms involving oxidative stress, altered steroidogenesis, and disruption of hypothalamic–pituitary–gonadal axis signaling, even at exposure levels that do not produce overt histopathological changes (17–20).

Sex-dependent differences further complicate the interpretation of endocrine responses to dietary additives. Male and female reproductive systems differ substantially in hormonal feedback mechanisms, steroid metabolism, and sensitivity to endocrine disruptors. Consequently, identical exposure is likely to provoke different hormonal responses in males and females (21–23). Artificial sweeteners, including Sod.S., have been increasingly demonstrated in experimental studies to induce sex-dependent divergent changes in estrogens, gonadotropins, and androgens, which might alter reproductive homeostasis even in the absence of evident pathology in reproductive organs (20, 22, 24).

Oxidative stress has been proposed as one of the mechanisms underlying sweetener-induced endocrine alterations (25). In this domain, vitamins with antioxidant properties, such as vitamins C and E, have attracted much research attention in relation to their known potent effects in achieving

a balance in oxidation states, as well as their well-known effects in enhancing reproduction (26–28). While Vitamin C functions as a potent water-soluble antioxidant within the aqueous compartments of the cell, Vitamin E acts as a critical lipid-soluble protector of cellular membranes. Both vitamins have been implicated in the regulation of gonadotropin secretion and testosterone synthesis under conditions of oxidative stress
(28, 30).

Antioxidant vitamins may exert differential and hormone-specific modulatory effects under conditions of chronic chemical exposure, potentially influencing endocrine responses beyond their classical protective roles (31–33).

While sodium saccharin is considered safe within its (ADI), emerging evidence suggests that endocrine modulation may occur independently of overt toxicity. However, chronic ADI-level exposure has not been adequately examined from a sex-comparative endocrine perspective. Furthermore, the selective and hormone-specific modulatory effects of antioxidant vitamins under such exposure conditions remain poorly defined. To address these gaps, the present study provides a comprehensive sex-dependent evaluation of reproductive hormonal alterations following prolonged ADI-level saccharin administration and systematically investigates the differential regulatory roles of vitamins C and E. This integrative approach offers novel insight into subtle endocrine disruption occurring within regulatory safety margins.

Materials And Methods

Animals

A total of 80 adult (3-4 months old) male and female Wistar rats weighing 200-325 g were kept in the animal house of the College of Veterinary Medicine, University of Duhok. The rats were kept in a well-ventilated room with controlled temperatures (22 ± 2°C) and normal light-dark cycles. Food and water were provided ad libitum. The handling and treatment of the rats followed the guidelines for the care and use of laboratory animals (34).

Chemicals

Sodium saccharin was obtained from Alfa Aesar Thermo Fisher Scientific (Germany); vitamin C was purchased from Scharlau (Spain); and vitamin E was sourced from Extrasynthese (France).

Experimental Design

Eighty adult male and female rats were split into four groups at random, ten male and ten female rats in each group. As the control group, Group 1 was given only distilled water. In accordance with the acceptable daily intake (ADI), Group 2 received 5 mg/kg.BW of Sod.S. (35). Group 3 received 5 mg/kg.BW of Sod.S. plus 150 mg/kg.BW of vitamin C (36), while group 4 received 5 mg/kg. BW Sod.S. combined with 100 mg/kg.BW of vitamin E (37). All treatments were given orally via gavage once daily for 90 days.

Collection of Samples

After an overnight fast, blood samples (3 mL) were withdrawn from retro-orbital plexus of all groups. Samples were obtained at two time points: at baseline (before treatment or 0 time) and after 90 days of administration. Blood was drawn into EDTA anticoagulant tubes, and the plasma was separated by centrifugation at 3000 rpm for 15 min.

The separated plasma was then transferred into plastic tubes; the tubes were stored at −20°C until hormonal analysis (38).

Hormonal Assays

Hormonal assays were conducted using a standard enzyme-linked immunosorbent assay (ELISA) kit (My BioSource, USA), specific for rats. The procedure was performed according to the manufacturer's instructions to determine plasma levels of estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone, based on the standard ELISA method (39).

Statistical Analysis

IBM SPSS software, version 22, was used to analyze the data using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. The statistical significance was set at $P < 0.05$. The mean ± standard error of the mean (SEM) for each group of ten rats is used to express the results. The formula (post-treatment - baseline)/baseline) × 100] was used to determine the percentage changes from baseline (40).

Results

Analysis of serum E2 concentrations after 90 days revealed significant differences between the study groups, as shown in Table 1 and Figure 1, Sod.S. increased E2 level by 52%, from (9.57 ± 0.56) at baseline to (14.62 ± 0.93) at 90 days, as compared to the control group, which showed a level of (9.69 ± 0.73) at 90 days. When Sod.S. was given in combination with vitamin C, it was found that the level of E2 was increased by about 22%, from (9.22 ± 0.52) at baseline to (11.27 ± 1.1) at 90 days, showing a marked difference as compared to the Sod.S. group alone. Moreover, the combination of Sod.S. and vitamin E showed an increase in the level of E2 by about 14%, from (8.68 ± 0.37) at the baseline to (9.93 ± 0.83) at 90 days relative to baseline, but remained lower than the Sod.S.-treated group.

In female rats, it was found that when treated with Sod.S., there existed a marked, significantly positive effect, as there was an increase in E2 by 42% from (45.94 ± 2.11), reaching (65.23 ± 3.60) after 90 days compared with those rats in the control group, who had E2 level values of (52.82 ± 3.11) after 90 days. On the contrary, rats treated with a combination of Sod.S. and vitamin C. resulted in a decrease in E2 levels compared to baseline and control, as there were reduced values

of E2 by 14% from (52.97 ± 2.450) with time, reaching (45.47 ± 2.28) after 90 days. Moreover, rats treated with a combination of Sod.S. and vitamin E showed an increased level of E2, as there was an elevation in E2 values by 25% from (48.47 ± 1.42) to (60.69 ± 3.29) after 90 days, a significant finding compared with those rats in the control group as well as those treated with Sod.S. only, as presented in Table 2 and Figure 1

Table 1: Effects of chronic sodium saccharin and antioxidant vitamins on reproductive hormone concentrations in male Wistar rats.

Parameter	Group	Baseline (Day 0)	90 Days	% Change
Estradiol (pg/mL)	Control	10.02 ± 0.68	9.69 ± 0.73	-3.29%
	Sod.S.	9.57 ± 0.56	14.62± 0.93[a]	52.77%
	Sod.S. + Vit C	9.22 ± 0.52	11.27 ± 1.10[b]	22.23%
	Sod.S. + Vit E	8.68 ± 0.37	9.93 ± 0.83[b]	14.40%
FSH (mIU/mL)	Control	2.83 ± 0.09	3.35 ± 0.27	18.37%
	Sod.S.	3.07 ± 0.18	2.40 ± 0.18[a]	-21.82%
	Sod.S. + Vit C	2.64 ± 0.17	2.62 ± 0.20[a]	-0.76%
	Sod.S. + Vit E	3.32 ± 0.28	4.39 ± 0.28[ab]	32.23%
LH (mIU/mL)	Control	8.89 ± 0.96	8.03 ± 0.31	-9.67%
	Sod.S.	9.49 ± 0.36	7.63 ± 0.28	-19.60%
	Sod.S. + Vit C	9.28 ± 0.39	6.77 ± 0.4[a]	-27.05%
	Sod.S. + Vit E	10.10 ± 0.31	8.79 ± 0.56	-12.97%
Testosterone (nmol/L)	Control	9.42 ± 0.31	10.13 ± 0.38	7.54%
	Sod.S.	9.21 ± 0.39	10.39 ± 0.86	12.81%
	Sod.S. + Vit C	8.95 ± 0.38	14.17 ± 0.66[ab]	58.33%
	Sod.S. + Vit E	9.95 ± 0.23	12.24 ± 0.41[ab]	23.01%

Note: Values are expressed as mean ± SEM (n = 10). Significance letters (a, b, ab) denote statistical differences at based on One-way ANOVA followed by Tukey's post-hoc test. Different superscript letters: a: vs. Control; b: vs. Sod.S. alone; ab: vs. both Control and Sod.S. groups.

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Table 2 Effects of chronic sodium saccharin and antioxidant vitamins on reproductive hormone concentrations in female Wistar rats.

Parameter	Group	Baseline (Day 0)	90 Days	% Change
Estradiol (pg/mL)	Control	50.02 ± 1.15	52.82 ± 3.11	5.60%
	Sod.S.	45.94 ± 2.11	65.23 ± 3.60[a]	41.99%
	Sod.S. + Vit C	52.97 ± 2.45	45.47 ± 2.28	-14.16%
	Sod.S. + Vit E	48.47 ± 1.42	60.69 ± 3.29[ab]	25.21%
FSH (mIU/mL)	Control	13.61 ± 1.70	14.16 ± 1.21	4.04%
	Sod.S.	14.09 ± 0.98	20.07 ± 1.0[a]	42.44%
	Sod.S. + Vit C	12.87 ± 0.55	15.33 ± 1.07[b]	19.11%
	Sod.S. + Vit E	13.12 ± 0.76	12.39 ± 0.83[ab]	-5.56%
LH (mIU/mL)	Control	7.56 ± 0.41	8.09 ± 0.24	7.01%
	Sod.S.	7.80 ± 0.39	10.03 ± 0.38[a]	28.59%
	Sod.S. + Vit C	7.65 ± 0.43	8.07 ± 0.58	5.49%
	Sod.S. + Vit E	8.04 ± 0.37	10.15 ± 0.45[ab]	26.24%
Testosterone (nmol/L)	Control	1.14 ± 0.05	1.20 ± 0.04	5.26%
	Sod.S.	1.22 ± 0.02	0.75 ± 0.05[a]	-38.52%
	Sod.S. + Vit C	1.24 ± 0.08	0.95 ± 0.06[b]	-23.39%
	Sod.S. + Vit E	1.06 ± 0.06	0.85 ± 0.09[ab]	-19.81%

Note: Values are expressed as mean ± SEM (n = 10). Significance letters (a, b, ab) denote statistical differences based on One-way ANOVA followed by Tukey's post-hoc test. Different superscript letters: a: vs. Control; b: vs. Sod.S. alone; ab: vs. both Control and Sod.S. groups.

In regards to FSH levels in males, Sod.S. administration significantly reduced FSH levels by approximately 22%, as shown in Table 1 and Figure 2, FSH levels in these animals dropped significantly from (3.07 ± 0.18) to (2.40 ± 0.18) by day 90, compared to the FSH level of (3.35 ± 0.27) of the control group at day 90. When Sod.S. was used in combination with vitamin C, FSH

level stability was noted, as FSH dropped from (2.64 ± 0.17) at baseline to (2.62 ± 0.2) at day 90, although still significantly higher than that of the control group

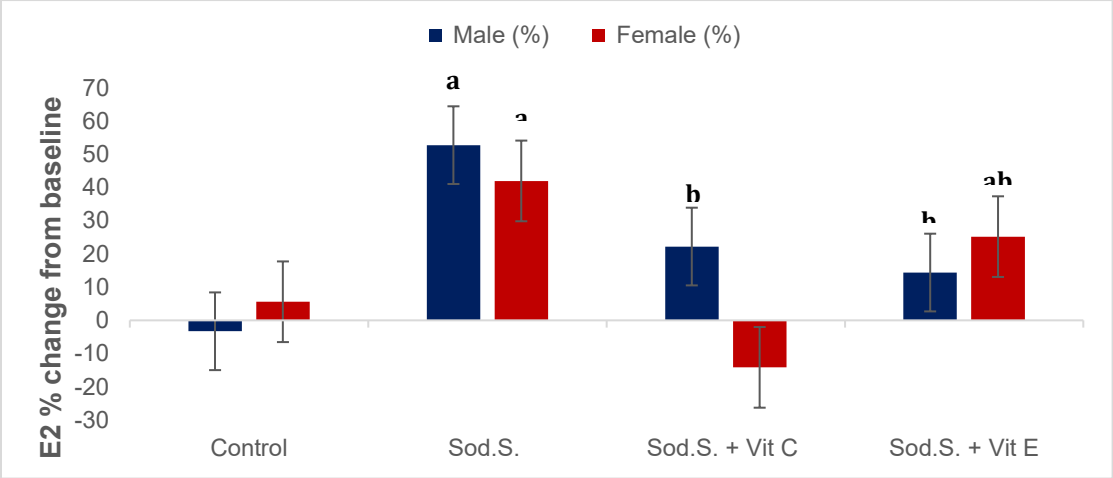

Figure 1. Percentage change in serum estradiol (E2) levels after 90 days of treatment relative to baseline (day 0) in male and female rats. Animals received sodium saccharin (Sod.S., 5 mg/kg b.w.) alone or in combination with vitamin C (150 mg/kg b.w.) or vitamin E (100 mg/kg b.w.). Data are expressed as percentage change from baseline. Error bars indicate the SEM (n=10). Significant differences (P < 0.05) are marked with letters; a: indicates a significant difference from the control group; b: indicates a significant difference from the Sod.S. only group; ab: indicates a significant difference from the both control and Sod.S. groups.

. However, vitamin E supplementation led to a significant increase in FSH level by approximately 32%, which increased from (3.32 ± 0.28) at baseline to (4.39 ± 0.28) at day 90. The female response was opposite to the male response, Sod.S. treatment administration alone was found to be significantly increase FSH levels by around 42% from (14.09 ± 0.98) at baseline to (20.07 ± 1.01) at 90 days when compared to the control, where the FSH level was (14.16 ± 1.21). FSH levels in the Sod.S. and vitamin C treated group increased by around 19% from (12.87 ± 0.55) at baseline to (15.33 ± 1.07) at 90 days, which was significant when compared to the Sod.S. treated group. FSH levels also decreased by around 6% from (13.12 ± 0.76) at baseline to (12.39 ± 0.83) at 90 days in the Sod.S. and vitamin E treated group, which was found to be significantly lower compared to the control and Sod.S. treated groups (Table 2, Figure 2).

Following the analysis of FSH, LH levels were evaluated. In male rats, Sod.S. alone did not show a statistically significant level of LH, which decreased from (9.49 ± 0.36) at baseline to (7.63 ±

0.28) at the end of the 90 days, and showed a non-significant decrease. The co-administration of Sod.S. and vitamin C was found to decrease the level of LH by a percentage of 27%, from (9.28 ± 0.39) at baseline to (6.77 ± 0.41) at the end of the 90 days when compared with the control group, whereas the co-administration of Sod.S. and vitamin E was found to decrease the level of LH from (10.1 ± 0.31) at baseline to (8.79 ± 0.56) at the end of the 90 days, although this increase was statistically insignificant (Table 1 Figure 3).

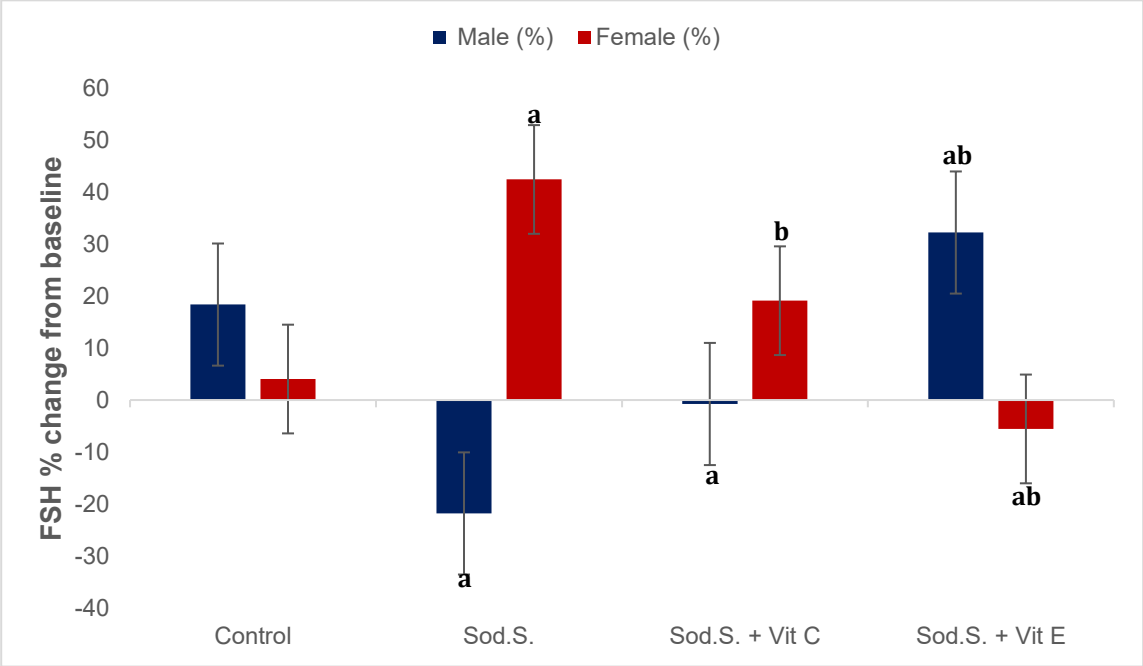

Figure 2. Percentage change in serum Follicle-Stimulating Hormone (FSH) levels after 90 days of treatment relative to baseline (day 0) in male and female rats. Animals received sodium saccharin (Sod.S., 5 mg/kg b.w.) alone or in combination with vitamin C (150 mg/kg b.w.) or vitamin E (100 mg/kg b.w.). Data are expressed as percentage change from baseline. Error bars indicate the SEM (n=10). Significant differences (P < 0.05) are marked with letters; a: indicates a significant difference from the control group; b: indicates: a significant difference from the Sod.S. only group; ab: indicates a significant difference from the both control and Sod.S. groups.

For female rats, Sod.S. significantly increased LH levels by approximately 29%, rising from (7.80 ± 0.39) at baseline to (10.03 ± 0.38) at 90 days, compared to the control group at 90 days, which had LH levels of (8.09 ± 0.24). Conversely, the group treated with Sod.S. combined with vitamin C did not significantly affect LH levels, as LH levels remained relatively stable from (7.65 ± 0.43) at baseline to (8.07 ± 0.58) at 90 days. However, the group receiving Sod.S. combined with vitamin E showed an increase in LH levels by approximately 26%, rising from (8.04 ± 0.37) at

baseline to (10.15 ± 0.45) at 90 days, which was significant when compared to both the control and Sod.S. treated groups. These results appeared in Table 2, Figure 3.

In addition, Sod.S. exposure alone in male rats had a negligible effect on male testosterone levels, with a slight increase from (9.21 ± 0.39) at baseline to (10.39 ± 0.86) at 90 days. However, the combination of Sod.S. with vitamin C resulted in a significant increase in testosterone levels by approximately 58%, rising from (8.95 ± 0.38) at baseline to (14.17 ± 0.66) at 90 days, which was significantly higher compared to both the control and Sod.S. treated groups. Similarly, the combination of Sod.S. with vitamin E led to a significant increase in testosterone levels by approximately 23%, from (9.95 ± 0.23) at baseline to (12.24 ± 0.41) at 90 days, also significantly higher compared to both the control and Sod.S. treated groups, as shown in Table 1 Figure 4

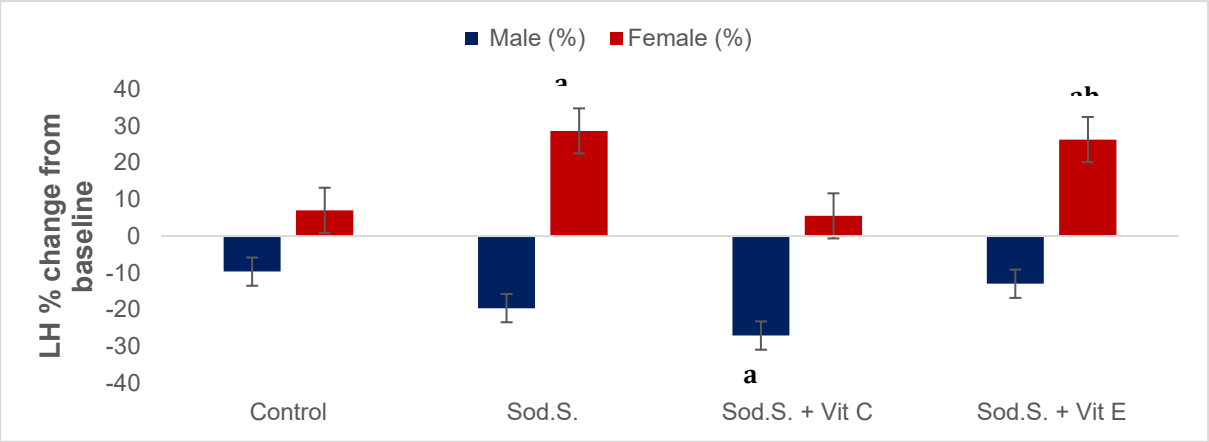

Figure 3. Percentage change in serum Luteinizing Hormone (LH) concentrations after 90 days of treatment relative to baseline (day 0) in male and female rats. Animals received sodium saccharin (Sod.S., 5 mg/kg b.w.) alone or in combination with vitamin C (150 mg/kg b.w.) or vitamin E (100 mg/kg b.w.). Data are expressed as percentage change from baseline. Error bars indicate the SEM (n=10). Significant differences (P < 0.05) are marked with letters; a: indicates a significant difference from the control group; b: indicates a significant difference from the Sod.S. only group; ab: indicates a significant difference from the both control and Sod.S. groups.

In female rats, treatment with Sod.S. alone resulted in a marked reduction in testosterone levels by 38%, which declined from a baseline of (1.22 ± 0.018) to (0.75 ± 0.046) by the end of the 90-day experiment; this change was significant compared to the control group. The co-administration of vitamin C with Sod.S. resulted in a notable 23 % decline in testosterone levels, which dropped from an initial mean of (1.24 ± 0.076) at baseline to (0.95 ± 0.058) at day 90. These results showed a significant difference when compared with the Sod.S. group. The group receiving a combination

of Sod.S. and vitamin E showed a substantial 19% decrease in testosterone, falling from (1.06 ± 0.045) at the start of the study to (0.85 ± 0.087) at 90 days. These results were statistically significant when compared with both the control and Sod.S. groups Table 2, Figure 4.

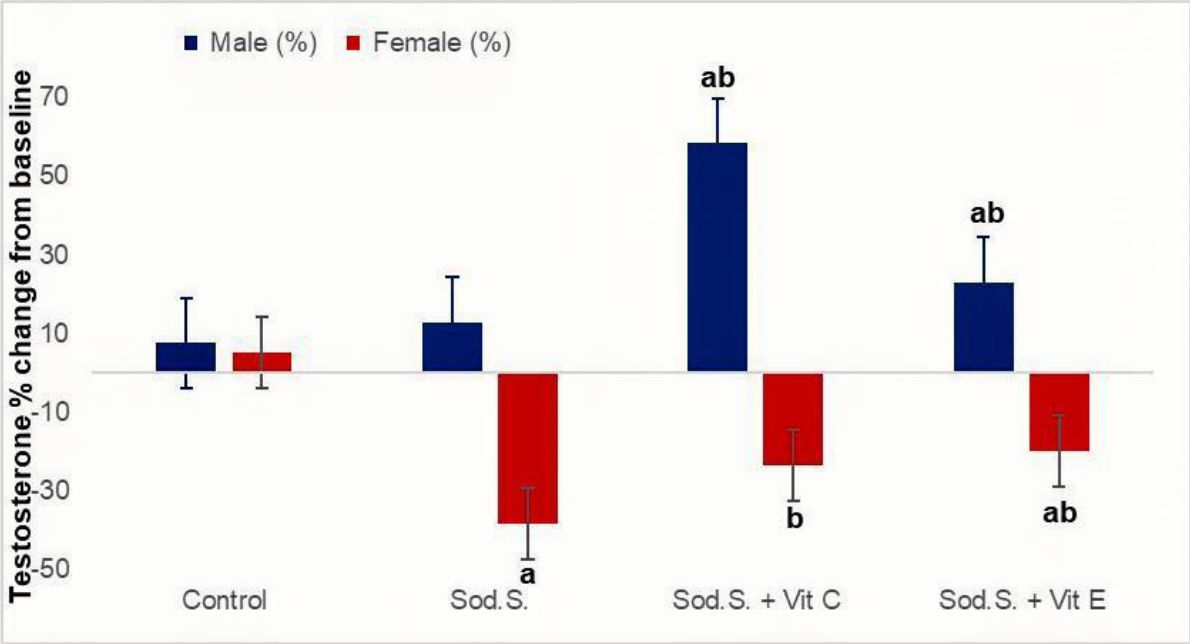

Figure 4. Percentage change in serum Testosterone Hormone concentrations after 90 days of treatment relative to baseline (day 0) in male and female rats. Animals received sodium saccharin (Sod.S., 5 mg/kg b.w.) alone or in combination with vitamin C (150 mg/kg b.w.) or vitamin E (100 mg/kg b.w.). Data are expressed as percentage change from baseline. Error bars indicate the SEM (n=10). Significant differences (P < 0.05) are marked with letters; a: indicates a significant difference from the control group; b: indicates a significant difference from the Sod.S. only group; ab: indicates a significant difference from the both control and Sod.S. groups.

Discussion

The results of this 90-day study indicate that chronic exposure to Sod.S. at the acceptable daily intake (ADI) level induces significant, sex-specific changes in the reproductive endocrine profile of Wistar rats. Notably, these alterations were observed in the absence of overt clinical signs of toxicity, suggesting that Sod.S. may exert endocrine-disrupting effects through non-classical mechanisms rather than through general systemic toxicity.

The current study clearly shows that chronic treatment with Sod.S. causes a significant change in E2 levels in both male and female rats. Notably, these changes were observed in the absence of overt toxicity, thus supporting the idea that endocrine disruption can occur independently of traditional toxicity (41). In male rats, the administration of Sod.S. caused a significant increase in

the level of circulating E2, whereas a similar but more dramatic increase was observed in females. These results are in line with previous studies indicating that artificial sweeteners may cause estrogenic or estrogen-modulating activities through interference with steroidogenesis or endocrine feedback regulation (17,20).

E2 up-regulation is significant as it indicates that long-term saccharin exposure can affect estrogen homeostasis in a manner suggesting potential relevance to human physiology. Previous studies suggest the possibility of interaction of non-nutritive sweeteners with estrogen receptors and/or alterations in the activity of aromatase enzymes, which would increase the levels of estrogen by accelerating estrogen synthesis and/or bioavailability (18,42). A greater response in females than in males involves sex differences in the sensitivity of ovarian steroidogenesis. In this context, estrogen feedback regulation in the hypothalamic-pituitary-gonadal axis also takes place (22,24).

The co-administration of vitamin C showed a modulatory effect in the saccharin-induced increase of E2 levels in males, whereas vitamin E showed a more significant normalization effect of E2 levels when compared with the saccharin-alone-treated group. The above findings conclude that antioxidant co-administration does not show antagonistic or inhibitory effects on hormonal imbalances but demonstrates a differential effect in the regulation of estrogen levels. Vitamin C, which is used in redox-sensitive enzymatic reaction systems, may exhibit a partial antagonistic or inhibitory effect in estrogen overdose production and regulation, whereas vitamin E, due to its lipid-dissolving properties, may show a more significant and effective regulation of steroidogenic processes and production of steroid hormones (28,43,44). The above findings also conclude that, in females, although vitamin E fails to normalize E2 levels, the regulation of E2 levels is subject to the influence of ovarian functions.

The observed effect of Sod.S. on FSH levels demonstrates a pronounced gender difference in the regulation of gonadotropins. In the male rats, the 22% decrease in FSH is most probably a consequence of the sharp increase in E2 levels. As previously discussed in the existing literature, the high levels of E2 induce a negative feedback response in the hypothalamic-pituitary-gonadal (HPG) axis, thereby indirectly instructing the anterior pituitary gland to reduce FSH secretion (45). This negative feedback response is consistent with the observation that artificial sweeteners can disturb endocrine communication by either affecting the gut microbiota or through gut microbiota alterations or endocrine signaling disruption in the process (18, 46). Since Sod.S. is metabolically stable compared to sucrose, these persistent metabolites could further contribute to oxidative stress, thereby further disrupting the intricate signaling process of the pituitary gland.

In contrast, the 42% spike in female FSH suggests a different physiological response—likely a compensatory mechanism. This surge may stem from the sweetener's interference with ovarian steroid synthesis or follicular regulation, forcing the HPG axis to over-adjust to maintain homeostasis (20, 25).

The role of antioxidant supplementation here was more modulatory than corrective. Vitamin C only partially stabilized FSH levels, supporting previous data that suggests it can buffer pituitary

function during chemical stress without fully restoring a baseline (47–49). However, vitamin E co-administration actually pushed male FSH levels above the control values. This suggests that vitamin E does not just "protect" the cells; it may actively enhance pituitary sensitivity to GnRH or reshape the communication pathways between the pituitary and the gonads (50-53).

Regarding the effect of Sod.S. was undoubtedly sex-dependent in rats with respect to its impact on the secretion of LH hormones. Indeed, the administration of Sod.S. was unable to bring any statistically significant impact on the release of LH hormones in male rats, whereas administration of the compound at ADI levels was able to induce increased levels of LH hormones in females. The impact of the compound appears more pronounced in females compared to males, possibly because of hypothalamic and ovarian responses (54,41).

The co-administration of Sod.S. with Vitamin C reduced LH levels in males, suggesting that antioxidant therapy may, in fact, affect LH levels indirectly by modulating redox-sensitive hypothalamic pathways (41). Female rats treated with Vitamin E supplementation elicited higher LH secretory levels compared to controls and to the saccharin-exposed group. This may be attributed to the fact that, under such a regimen, hypothalamic-pituitary and steroidogenic functions exhibit fewer oxidative stress (28,41). These observations, again, promote the argument that antioxidants act selectively on hormones and have a sex-specific action.

Further changes in testosterone support regulatory effects, showing clear patterns of differences between sexes and treatment groups. In males, administration of Sod.S. did not significantly impact testosterone levels independent of treatment, indicating that ADI-level exposure does not directly suppress Leydig cell steroidogenesis. This contrasts with reports at higher dosing of saccharin, where testosterone suppression has been observed, further underscoring the sensitivity of dose selection in endocrine studies (20,55).In females, however, saccharin exposure was associated with a significant decrease in testosterone levels. This decrease might relate to altered ovarian theca cell activity or disrupted androgen-to-estrogen conversion, as has been suggested previously regarding artificial sweetener effects on female steroidogenesis (24,56). Such changes may have implications for follicular development and reproductive cyclicity. The supplementation of vitamin C caused the most significant rise in testosterone levels in male rats, a finding which was supported by evidence showing that this vitamin enhances testosterone synthesis and protects steroidogenic enzymes under oxidative stress conditions (29,57). Vitamin E also raised levels of the androgen, but to a lesser degree, suggesting a complementary role but one involving a different mechanism of action. Vitamin E might maintain the membrane integrity of Leydig and theca cells, thus preserving steroidogenic capacity, whereas vitamin C might be required to support enzymatic activity directly involved in testosterone biosynthesis (58–60). These results further reinforce the idea that antioxidant vitamins can modulate endocrine responses in an antioxidant-specific and hormone-specific manner. Although these findings are of great interest, only one dose of Sod.S. at the ADI level has been tested; consequently, it is not possible to determine the dose-response relationship. Additionally, oxidative stress biomarkers, steroidogenic enzyme expression, and histopathological

examinations were not assessed, limiting mechanistic interpretation. Estrous cycle staging in female rats was also not controlled. The estrous cycle of female rats was not controlled, which may affect their hormonal status. Further research is required to understand the mechanisms of endocrine disruption by sodium saccharin, with molecular changes as endpoints

Conclusion

Significant alterations in reproductive hormone levels in male and female rats were observed when the acceptable daily intake dose of Sod.S. was administered chronically, indicating that endocrine modulation can be achieved even in the absence of toxicity. The data were sex-specific, as hormone levels are differentially regulated in males and females. The combined effect of vitamins C and E was not similar for endocrine modulation; instead, each vitamin had a different modulating effect on various hormones. Taken together, these results suggest that chronic consumption of Sod.S. may have a subtle effect on the balance of reproductive hormones and that antioxidants have a differential modulating effect on these hormonal changes.

Ethical approval

For the study was granted by the Animal Ethics Committee at the College of Veterinary Medicine, University of Duhok, Iraq (Ethical code No. DR1996919CV, approved on the 11th of June, 2019).

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Conflict of Interest

The author declares that they have no conflict of interest.

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التأثيرات المعتمدة على الجنس للصوديوم سكارين والفيتامينات المضادة للأكسدة على الهرمونات التناسلية في جرذان ويستار عند مستويات الجرعة اليومية المقبولة

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الخلاصة

تثير المُحلّيات غير المغذية، بما في ذلك سكرين الصوديوم (Sodium Saccharin)، مخاوف متزايدة بسبب تأثيراتها المحتملة المُسببة لاضطرابات الغدد الصماء على الصحة الإنجابية. صُمِّمَت هذه الدراسة لبحث تأثيرات سكرين الصوديوم على مستويات الهرمونات التناسلية في ذكور وإناث جرذان (Wistar) "، وكذلك لتقييم الدور الوقائي للفيتامينين (ج) و(هـ) كمضادات للأكسدة. تم تقسيم ثمانين جرذاً بالغاً عشوائياً إلى أربع مجموعات: مجموعة السيطرة (المجموعة الاولى)؛ مجموعة عولجت بسكرين الصوديوم بجرعة 5 ملغم/كغم من وزن الجسم (المجموعة الثانية)؛ مجموعة الصوديوم سكرين مع فيتامين ج بجرعة (150 ملغم/كغم) (المجموعة الثالثة)؛ مجموعة الصوديوم سكرين مع هـ بجرعة (100 ملغم/كغم) (المجموعة الرابعة)؛. تم إعطاء العلاجات عن طريق الفم لمدة 90 يوماً، تلاها قياس مستويات الإستراديول(E2) ، الهرمون المنبه للجريب(FSH) ، الهرمون اللوتيني(LH) ، والتستوستيرون في بلازما الدم. أظهرت النتائج في الذكور أن الصوديوم سكرين تسبب في ارتفاع مستوى الإستراديول بنسبة 52٪ وانخفاض مستوى الهرمون المنبه للجريب بنسبة 22٪ مقارنة بمجموعة السيطرة. بينما أدى الإعطاء المشترك لفيتامين ج إلى زيادة إضافية في مستوى الإستراديول بنسبة 22٪ (مقارنة بمجموعة الصوديوم سكرين وحدها) ورفع التستوستيرون بنسبة 58٪ (مقارنة بكل من مجموعتي السيطرة والصوديوم سكرين). في حين أدت المعالجة بفيتامين هـ إلى ارتفاع مستوى الهرمون المنبه للجريب بنسبة 32٪ والتستوستيرون بنسبة 23٪ مقارنة بمجموعتي السيطرة والصوديوم سكرين. وفي إناث الجرذان، أدى الصوديوم سكرين إلى زيادة الهرمون المنبه للجريب بنسبة 42٪، والإستراديول بنسبة 42٪، والهرمون اللوتيني بنسبة 29٪، في حين انخفض التستوستيرون بنسبة 38٪ مقارنة بمجموعة السيطرة. أدت المعالجة بسكرين الصوديوم مع فيتامين ج إلى زيادة مستوى الهرمون المنبه للجريب بنسبة 19٪ وانخفاض التستوستيرون بنسبة 23٪ مقارنة بمجموعة السيطرة. أما فيتامين هـ فقد أظهر قدرة فائقة في استعادة توازن موجهات الغدد التناسلية .تستنتج الدراسة أن سكرين الصوديوم يعطل المحور الهرموني التناسلي بطرق تعتمد على الجنس؛ حيث يؤدي إلى تأثيرات إستروجينية في الذكور وحالة من فرط موجهات الغدد التناسلية في الإناث. عمل فيتامين هـ بفعالية على تحسين توازن الهرمونات المنبهة للغدد التناسلية، بينما أظهر فيتامين ج تأثيراً أكبر على مستويات التستوستيرون، مما يشير إلى وجود آليات وقائية متفاوتة للمكملات المضادة للأكسدة.

الكلمات المفتاحية: الصوديوم سكرين، الفيتامينات المضادة للأكسدة، اضطرابات الغدد الصماء، جرذان، الهرمونات التناسلية