Int. J. Morphol.,
44(2):542-554, 2026.

Eminent Effects of Glucagon-Like Peptide-1 Receptor Agonist on the Thyroid Gland of Male Albino Rats

Efectos Notables del Agonista del Receptor del Péptido Similar al Glucagón-1 en la Glándula Tiroides de Ratas Albinas Macho

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SALAMA, R. M.; ESSAWY, A. S. & OMAR, M. A. Eminent effects of glucagon-like peptide-1 receptor agonist on the thyroid gland of male albino rats. *Int. J. Morphol., 44(2)*:542-554, 2026.

SUMMARY: Effects of Glucagon-like peptide-1 receptor agonists on the thyroid gland, particularly the parafollicular C-cells, have been noted for injectable formulations, but comprehensive data regarding the novel oral formulation of semaglutide remain limited. The aim of the study was to investigate and quantify the dose-dependent effects of chronic oral semaglutide administration on the endocrine function, morphology, and cellular markers of proliferation and apoptosis in the thyroid gland of male albino rats. Forty (40) adult male rats were divided into 4 groups; control and 3 semaglutide treatment groups (Low-Dose, Medium-Dose, and High-Dose), corresponding to the human therapeutic range of 3 to 14 mg, and treated for 30 days. Endocrine status was assessed via serum levels of tri-iodothyronine (T3(, thyroxine (T4(, and thyroid stimulating hormone (TSH). Histological analysis included Hematoxylin and Eosin (Hx&E) staining, Periodic Acid Schiff (PAS) reaction, and immunohistochemical (IHC) quantification of Ki67, Calcitonin, and BAX. All treated groups exhibited a significant, dose-dependent reduction in body weight. The Medium- Dose and High-Dose groups showed a highly significant decline ($P<0.001$) in all 3 measured serum thyroid hormones. Pathological examination revealed dose-proportional deterioration, including follicular atrophy and epithelial exfoliation. Immunohistochemistry confirmed a highly significant, dose-dependent increase in calcitonin immunoreactive C- cells numbers and Ki67 and BAX expression in the higher-dose groups ($P< 0.001$). Oral semaglutide induces notable and dose-dependent detrimental effects on the morphological and endocrine integrity of the rat thyroid gland, particularly at doses equivalent to 7 and 14 mg in humans. This mandates further research to delineate the underlying molecular mechanisms.

KEY WORDS: Semaglutide; TSH; Ki67; Calcitonin; BAX.

INTRODUCTION

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) represent a novel class of therapeutics that have profoundly revolutionized the clinical management of type 2 diabetes (T2D) because of their efficacy in lowering blood glucose, promoting weight loss, and offering cardiovascular and renal benefits (Gogineni *et al*., 2024). The incretin metabolic hormone, glucagon-like peptide-1 (GLP-1), is fundamentally synthesized and released by L-cells residing in the small and large intestine, as well as by specific cells within the central nervous system (Campbell & Drucker, 2013). This hormone is centrally accountable for orchestrating a diverse array of crucial physiological effects, including the control of glucose homeostasis and appetite regulation, which are mediated through GLP-1 receptors extensively distributed in organs such as the brain, kidneys, lungs, cardiovascular system, and gastrointestinal tract (Pyke *et al*., 2014). Since 2005, a growing number of GLP-1 RAs—including exenatide, liraglutide, lixisenatide, dulaglutide, and semaglutide—have successfully received regulatory authorization for the therapeutic intervention of T2D (Amaro *et al*., 2022).

Exenatide (Byetta®, Amylin Pharmaceuticals, LLC) was the first glucagon-like peptide-1 receptor agonist (GLP-1RA) introduced for clinical use as an adjunct therapy in individuals with type 2 diabetes (T2D) (Barnett, 2012). Subsequently, liraglutide (Victoza®, Novo Nordisk) was developed and approved for similar therapeutic purposes (Flint *et al*., 2011). However, both exenatide and liraglutide—the earliest GLP-1RAs—have been associated with the occurrence of thyroid C-cell tumors in rodent models following prolonged exposure (Madsen *et al*., 2012). This phenomenon has been further supported by studies demonstrating that prolonged liraglutide exposure

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Received: 2025-12-27 Accepted: 2026-02-08

specifically stimulates the release of plasma calcitonin, concurrently increasing calcitonin gene expression and consequently inducing C-cell hyperplasia in rat models (Knudsen *et al*., 2010).

While GLP-1 receptor agonists are known for their profound metabolic effects, their impact on the thyroid gland, particularly the parafollicular C-cells, remains a significant area of research. The GLP-1 receptor is notably expressed on rodent thyroid C-cells, which explains the observed C-cell hyperplasia and neoplasia in rats and mice and the resulting elevated calcitonin secretion following treatment with GLP-1 RAs models (Knudsen *et al*., 2010).

Prolonged GLP-1RA activation is consistently linked to elevated calcitonin levels and the development of tumors in rats and mice (Madsen *et al*., 2012). Conversely, the clinical evidence in humans is considerably less conclusive, often showing a decrease in TSH levels without a significant corresponding change in free T4 or T3 (Lisco *et al*., 2023). The precise mechanisms by which GLP-1 receptor activation alters thyroid function parameters and hormone levels are currently under active investigation.

The recently developed oral semaglutide (Rybelsus®) represents the first GLP-1RA available in an oral formulation approved by the U.S. Food and Drug Administration (Choe & Cho, 2021). It was approved for T2D management and, in 2021, for chronic weight control in overweight or obese individual (Gibbons *et al*., 2021). Semaglutide incorporates the absorption enhancer sodium N-[8-(2-hydroxybenzoyl) amino] caprylate to improve bioavailability (Husain *et al*., 2019). It has also demonstrated significant cardiovascular benefits (Pratley *et al*., 2019).

Furthermore, oral semaglutide shows superior efficacy in body weight reduction compared with dulaglutide, liraglutide, and other GLP-1RAs (Yabe *et al*., 2020), and exhibits remarkable glycemic and weight control outcomes in the PIONEER trials (Andersen *et al*., 2021).

Oral semaglutide is approved for clinical use at doses of 3, 7, and 14 mg. In the PIONEER 1 trial involving 703 patients with T2D, the 7 mg and 14 mg doses significantly reduced HbA1c levels at 6.5 months and body weight after 13 months of treatment (Anderson *et al*., 2020). Moreover, in participants consuming high-fat, high-calorie breakfasts, 14 mg oral semaglutide administered for three months reduced energy intake and body fat percentage, accompanied by increased fullness and satiety (Gibbons *et al*., 2021).

Recent evidence has highlighted potential interactions between glucagon-like peptide-1 receptor agonists (GLP-1RAs) and thyroid function. Köseoglu *et al*. (2020) reported that exenatide therapy reduced thyroid volume and modulated TSH levels in patients with type 2 diabetes, suggesting a direct or indirect influence on thyroid physiology. Ruska *et al*. (2024) demonstrated that GLP-1 receptor signaling exerts differential effects on thyroid follicular and parafollicular cells, indicating tissue-specific receptor expression and responses. Similarly, a 2024 review by Capuccio *et al*. (2024), in Biomolecules summarized that chronic GLP-1RA exposure may alter thyroid hormone synthesis, cellular proliferation, and calcitonin secretion, although results remain inconsistent across species and compounds.

Based on these observations, we hypothesized that oral semaglutide, a novel GLP-1RA formulation, may influence thyroid hormonal balance and morphology in a dose-dependent manner. Specifically, we proposed that semaglutide administration could alter serum T3, T4, and TSH levels and induce histological and immunohistochemical changes in the thyroid gland of rats, potentially reflecting underlying proliferative and apoptotic mechanisms.

Given the established, but not fully elucidated, thyroid-related effects of injectable GLP-1 RAs in rodent models, particularly concerning C-cell proliferation and the lack of comprehensive data on the oral form, this study was established with a specific, testable hypothesis: Chronic administration of the oral GLP-1RA semaglutide would induce dose-dependent alterations in thyroid hormone levels, follicular morphology, and C-cell proliferation/apoptosis markers in male albino rats, consistent with an effect mediated by the GLP-1 receptor distribution in rat thyroid tissue and potentially secondary metabolic alterations.

This investigation therefore aimed to evaluate the effects of the recently developed oral version of GLP-1RA semaglutide on the endocrine and morphological parameters of the thyroid gland of male albino rats.

MATERIAL AND METHOD

Animals and Ethics

A total of 40 adult male albino rats, weighing between 150–180 grams, were obtained and housed at the designated animal facility within the Faculty of Medicine, Menoufia University. The rats were maintained in specific rooms with unrestricted access to standard rodent chow and drinking water. Following a one-week acclimatization period in the cage.

Ethical approval. The experimental protocol was formally initiated. Ethics clearance was obtained from the institutional scientific research ethics committee (IRB approval number and date 8/2025ANAT7-1) of Menoufia University's Faculty of Medicine.

Chemicals and Dosage Preparation

Oral semaglutide (Rybelsus®) was procured from a local pharmacy (Novo Nordisk, Copenhagen, Denmark). The dosages administered to the rats were precisely calculated to be equivalent to the human therapeutic dosages of 3 mg, 7 mg, and 14 mg. Based on an assumed common human body weight of 60 kg, the calculated equivalent rat dosages were: 0.05 mg/kg (equivalent to 3 mg human dose), 0.12 mg/kg (equivalent to 7 mg human dose), and 0.23 mg/kg (equivalent to 14 mg human dose). A pill crusher was utilized to finely mill a fresh oral semaglutide tablet into a powder, which was subsequently dissolved in distilled water (DW) and manually shaken 8–10 times prior to oral administration. The refeeding schedule for rats was as follows: food was withdrawn at 4:00 p.m. and water at 6:00 p.m.; oral semaglutide was administered at 7:30 p.m., and both food and water were reintroduced from 8:00 p.m. to 4:00 p.m. the following day. This refeeding protocol was maintained for thirty consecutive days in each group (Rakhat *et al*., 2023).

Experimental Design and Group Nomenclature

For enhanced clarity and consistent reporting across the manuscript, Figures, and Tables, descriptive labels were used.

The 40 adult male albino rats were systematically assorted into 4 groups (n=10 rats each) with respect to semaglutide dose throughout the 30-day experiment, as follows:

1. Group I (Control Group): Rats received 0.5 mL of distilled water (DW).
2. Group II (Low-Dose Semaglutide Group): Rats received 0.05 mg/kg oral semaglutide.
3. Group III (Medium-Dose Semaglutide Group): Rats received 0.12 mg/kg oral semaglutide.
4. Group III (High-Dose Semaglutide Group): Rats received 0.23 mg/kg oral semaglutide.

Evaluation Methods

Rat weight (RW) Analysis: Rat body weights were precisely recorded three times throughout the 30-day experiment, with measurements taken once every 10 days to track temporal changes.

Hormonal Biochemical Analysis: At the conclusion of the 4th week, blood samples were collected via cardiac puncture from the left ventricle under anesthesia. Serum was immediately extracted and utilized to estimate the circulating levels of tri-iodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH). The assays were performed using the Milliplex Map rat thyroid hormone TSH panel-2 plex (EMD Millipore, Billerica, MA, USA).

Histological and Immunohistochemical Assessments

At the conclusion of the experiment, the thyroid glands were gently dissected (Hadie *et al*., 2013) and processed for light microscopic histological and immunohistochemical analyses. Thyroid specimens were fixed in 10 % neutral buffered formalin, processed, and embedded in paraffin.

A. Histological Study, Sections (5 μm) were stained with:

· Hematoxylin and Eosin (Hx & E) for general morphology assessment.
· Periodic Acid Schiff (PAS) for the detection and assessment of glycoprotein content in the colloidal material.

B. Histological Alterations, including colloid depletion, vascular congestion, and exfoliation of follicular epithelium, were semi-quantitatively scored on a scale of 0–3 in ten randomly selected high-power fields per section (0 = none, 1 = mild, 2 = moderate, 3 = severe).

C. Immunohistochemical (IHC) Study

· Ki67 (cell proliferation marker): Antigen retrieval of deparaffinized sections was performed using citrate buffer (pH 6.0) in a microwave, followed by incubation with rabbit anti-Ki67 monoclonal antibody for 30 minutes (Thermo Fisher Scientific, USA; Cat. No. RM-9106-R7) after treatment with 3 % hydrogen peroxide to block endogenous peroxidase activity. Antibody binding was visualized using diaminobenzidine. Negative controls underwent the same procedure without the primary antibody, while tonsil tissue served as the positive control. Ki67 immunoreactivity was identified as brown nuclear staining in positive cells (Matsuu-Matsuyama *et al*., 2015).
· Calcitonin (parafollicular cell marker): Detection was performed using a rabbit polyclonal anti-calcitonin antibody (DAKO A-576; Dako, Glostrup, Denmark). Rabbit/mouse immunoglobulins (Life Trade, Egypt) were applied as the secondary antibody, and 3,3-diaminobenzidine tetrahydrochloride served as the chromogen. Mayer's hematoxylin was used for counterstaining (Martín-Lacave *et al*., 2009).
· BAX (apoptotic marker): Mouse anti-Bax monoclonal

antibody (Ab-14 Golden, Lab Vision Clone B-9; Santa Cruz Biotechnology Inc., USA) was used as the primary antibody. Hodgkin's lymphoma tissue served as the positive control. Positive cytoplasmic staining was indicated by a brown coloration, and Mayer's hematoxylin was used for counterstaining (Van Noorden & Polak, 2014).

Morphometrical Studies and Quantification

All measurements were quantified in 5 distinct sections per rat and averaged across 5 high-power fields (x400) for each group using the Leica Q 500 MC image analyzer software.

1. **Qualitative Histological Analysis (Semi-Quantitative Scoring).** For rigorous assessment of histopathological alterations, a semi-quantitative scoring system was systematically applied to the Hx&E-stained sections. This approach was employed to provide quantitative support for the observed qualitative changes by classifying the severity and prevalence of key pathological features: vascular congestion, colloid reduction/empty lumen, and follicular epithelial exfoliation. The resulting scores facilitated objective reporting of both the inter-sample consistency (the frequency of the finding across all ten animals in a group) and the intra-sample consistency (the prevalence of the finding within standardized microscopic fields).
2. **Quantitative Histological Analysis of follicle diameter (μm)** was quantitatively measured using the Leica Q 500 MC image analyzer software on H&E-stained sections at a magnification of x400.
3. **Quantitative IHC Analysis (Ki67, Calcitonin, and BAX)**. Image analysis was conducted using ImageJ (NIH, USA). For each marker, five non-overlapping fields per section were analyzed. The immunoreactive area % of DAB-positive staining was measured using color deconvolution and thresholding techniques and expressed as the fraction of positive signal relative to the total field area.

The primary metric utilized for all three immunohistochemical markers was the area % of positive immunoreactive cells on immune-stained sections (magnification x 400). To ensure objective and accurate quantification of the chromogen deposition, the specific brown stain indicative of positive immunoreactivity was isolated, thresholded, and measured by applying a color deconvolution algorithm within the image analysis software. This standardization across markers facilitated consistent and accurate analysis.

Statistical Analysis

Data was analyzed using SPSS version 27.0. The mean, standard deviation (SD), and range were determined. Comparisons between each pair of groups were performed using the Mann-Whitney U test. A P-value of < 0.05 was deemed statistically significant. Given the multiple pairwise comparisons performed across the 4 groups, a Bonferroni correction or an alternative multiple comparison adjustment must be applied to the post-hoc Mann-Whitney U tests to rigorously control for the overall Type I error rate (family-wise error rate). For data presentation, time-course data will be visualized with line graphs, and single-point data will be presented using vertical scatter plots (dot plots) with error bars (SD) and statistical notation to clearly indicate significant differences.

Data were expressed as mean ± SD. Statistical comparisons between groups were performed using the Kruskal–Wallis's test, followed by Mann–Whitney U post-hoc tests with Bonferroni correction for multiple comparisons. Differences were considered significant at $p < 0.05$.

RESULTS

Rat weight (RW) Results

The chronic, 30-day administration of oral semaglutide induced statistically significant, dose-dependent body weight reduction in male albino rats compared to the Control Group. Weight measurements, recorded every 10 days to monitor the temporal trend, are systematically detailed in Table I and

Table I. Rat weight/gm among the study groups.

	Group I (N = 10)	**Group II** (N = 10)	**Group III** (N = 10)	**Group IV** (N = 10)	**U test**	**P value**
Rat weight/gm (I)					2.79	0.007[1]
Mean ±SD	180.5±2.5	175.2±6.2	163.9±1.3	152.7±1.6	3.83	<0.001[2]
Range	178 – 187	166 – 186	163 – 166	150 - 155	3.95	<0.001[3]
Rat weight/gm (II)					2.77	0.006[1]
Mean ±SD	181.6±3.9	175.9±3.8	159.4±2.8	143.7±3.7	3.79	<0.001[2]
Range	177 – 188	169 – 180	156 – 166	139 – 150	3.85	<0.001[3]
Rat weight/gm (III)					3.07	0.002[1]
Mean ±SD	191.0±5.8	180.4±6.9	156.0±7.6	137.2±6.1	3.78	<0.001[2]
Range	182- 200	166- 186	148 – 169	130 – 145	3.95	<0.001[3]

U = Mann Whitney U test. 1: Comparing group I and group II. 2: Comparing group I and group III. 3: Comparing group I and group IV

visualized in Figure 1. The Low-Dose Semaglutide Group demonstrated a statistically significant decrease in mean body weight throughout the observation period (e.g., P = 0.007 at the initial 10-day interval). Furthermore, both the Medium-Dose Semaglutide Group and the High-Dose Semaglutide Group exhibited a highly significant decline (P < 0.001) in body weight compared to the Control Group, thereby confirming the expected dose-dependent weight-reducing efficacy of the agent.

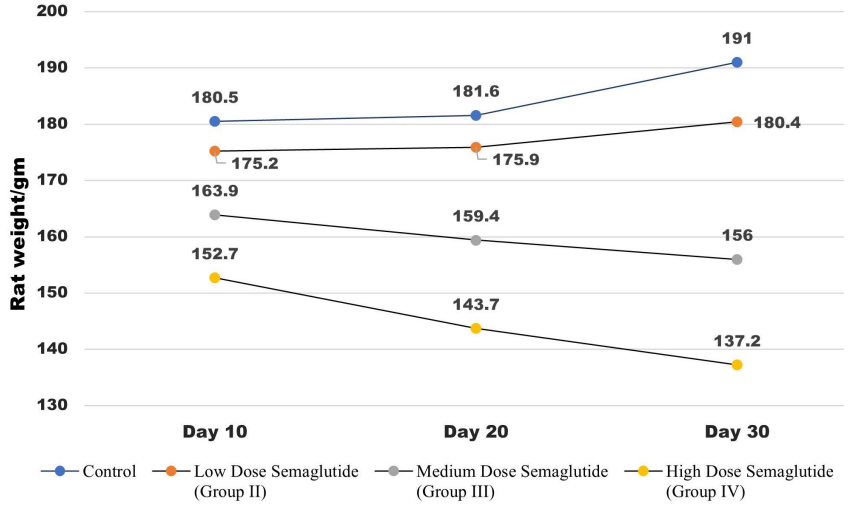

Fig. 1. Comparison of Rat Weight (gm) among the study groups over the time course of 30-Day experimental period.

Hormonal Biochemical Results

Serum hormonal assays for thyroid function revealed a clear dose-dependent impact. The Low-Dose Semaglutide Group showed only a non-significant variation compared to the Control Group for tri-iodothyronine (T3) serum level (P = 0.12), thyroxine (T4) (P = 0.08), and thyroid-stimulating hormone (TSH) (P = 0.1). However, the therapeutic effect intensified dramatically in the higher dose cohorts; both the Medium-Dose Semaglutide and the High-Dose Semaglutide Groups demonstrated a highly significant reduction (P < 0.001) in the circulating serum concentrations of T3, T4, and TSH when compared to the Control Group (Table II, Fig. 2).

Table II. Hormonal biochemical analysis among the study groups.

	Group I (N = 10)	Group II (N = 10)	Group III (N = 10)	Group IV (N = 10)	Test	P value
T3					1.55	0.12[1]
Mean ±SD	3.90 ±0.16	3.76±0.20	2.92±0.17	2.11±0.43	3.71	<0.001[2]
Range	3.60 – 4.15	3.38 – 4.01	2.7 – 3.33	1.5 – 2.77	3.99	<0.001[3]
T4					1.71	0.08[1]
Mean ±SD	4.01±0.14	3.78±0.22	3.06±0.28	2.12±0.45	3.66	<0.001[2]
Range	3.66 – 4.15	3.41 – 4.01	2.74 – 3.51	1.6 – 2.86	4.05	<0.001[3]
TSH					1.67	0.10[1]
Mean ±SD	0.93±0.17	0.82±0.10	0.16±0.09	0.08±0.04	3.66	<0.001[2]
Range	0.67 – 1.2	0.65 – 0.93	0.06 – 0.33	0.02 – 0.14	4.10	<0.001[3]

U = Mann Whitney U test. 1: Comparing group I and group II. 2: Comparing group I and group III.3: Comparing group I and group IV.

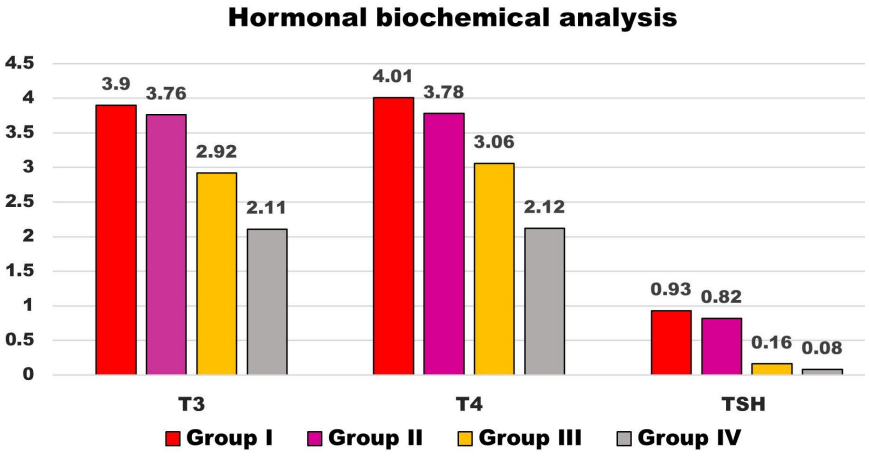

Fig. 2. Hormonal biochemical analysis of T3, T4, and TSH in the study groups.

Histological, Immunohistochemical, Morphometrical and Statistical Results

1. Qualitative Histological Analysis (Semi-Quantitative Scoring). Histological examination confirmed a progressive, dose-dependent pathological deterioration of the thyroid gland structure.

Control group: Sections stained with Hx&E show normal thyroid glands histological structure. They consist of follicles of variable diameters lined

with cuboidal follicular epithelium with rounded nuclei. Follicular lumen is filled with homogenous acidophilic colloid showing peripheral tiny vacuoles. There are a few interfollicular cells visible between the follicles (Fig. 3A). PAS-stained sections of the control group demonstrated an intense positive reaction (Fig. 4A).

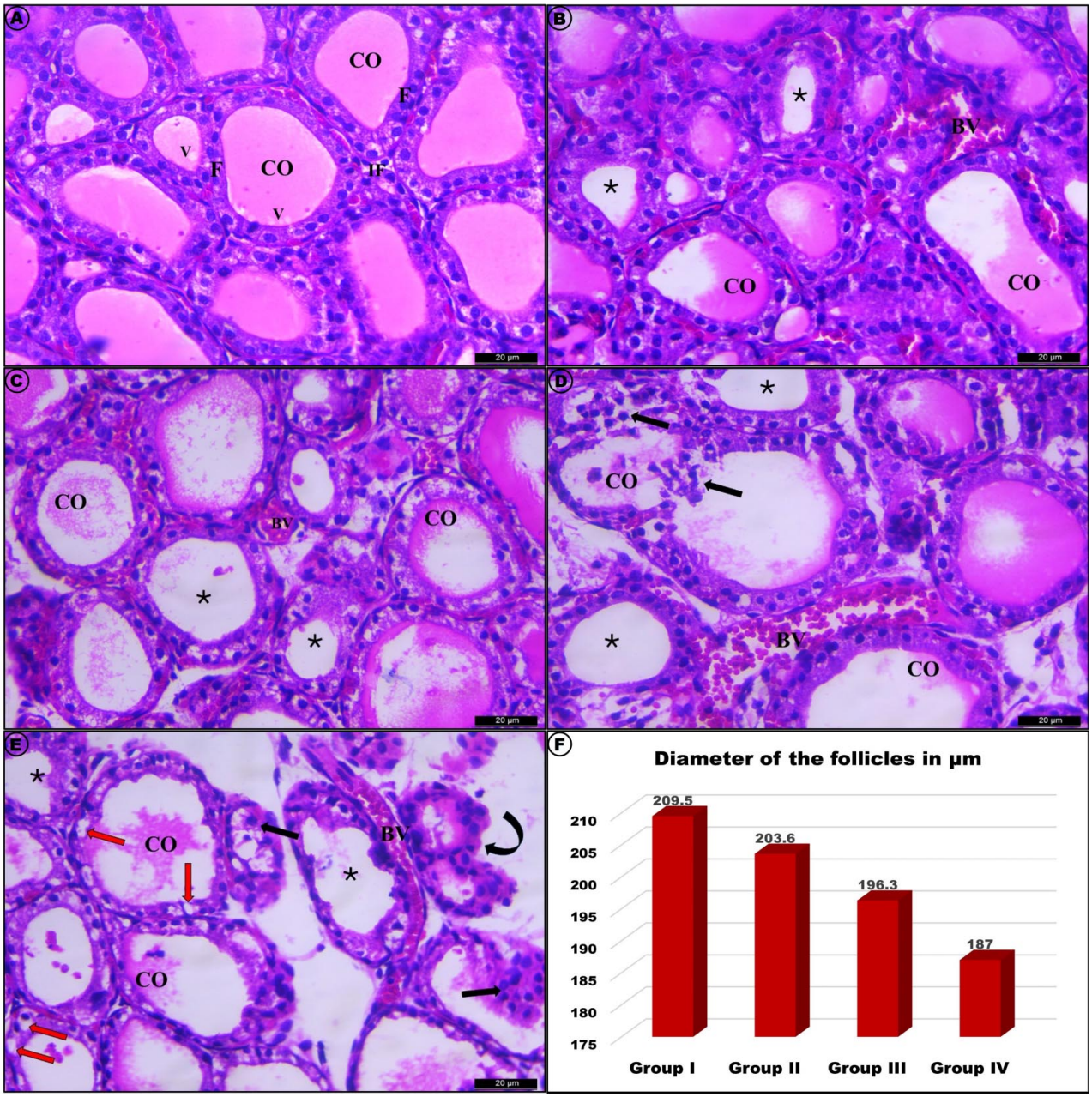

Fig. 3. Histopathological changes and follicle diameter in the thyroid gland (Hx&E, x400). **A.** A photomicrograph of group I reveals normal thyroid follicles lined with cuboidal follicular epithelium (F) with rounded nuclei. The follicular lumen is filled with homogenous acidophilic colloid (CO) shows peripheral small vacuoles (V). Some interfollicular cells (IF) can be seen in-between the follicles. **B**. A photomicrograph of group II shows a congested blood vessel (BV). Some follicles show a decrease in colloidal material (CO), others appear with empty lumen (Asterix). **C.** A photomicrograph of group III reveals a congested blood vessel (BV). Most follicles show more reduction in the colloidal material (CO), others appear with empty lumen (Asterix). **D.** A photomicrograph of group IV displays exfoliated follicular epithelium (black arrows), a congested blood vessel (BV). Most follicles show reduction in the colloidal material (CO), others appear with empty lumen (Asterix). **E.** Another photomicrograph of group IV reveals distorted follicles (arched arrow), exfoliated follicular epithelium (black arrows), follicular vacuolation (red arrows), a congested blood vessel (BV). Most follicles show reduction in the colloidal material (CO), others appear with empty lumen (Asterix). **F.** Histogram shows the mean diameter of the follicles in µm among the study groups.

Low-Dose Semaglutide and Medium-Dose Semaglutide Groups: Sections from these groups presented with congested blood vessels, a decrease in colloidal material in some follicles, and the appearance of empty lumina in others (Figs. 3B,C). These findings were supported by a moderate (Low-Dose) and mild (Medium-Dose) positive PAS staining reaction, respectively, indicating a diminishing thyroglobulin content (Figs. 4B,C).

High-Dose Semaglutide Group: This group displayed the most pronounced structural damage, evidenced by severe features such as exfoliated follicular epithelium, follicular vacuolation, and severe vascular congestion (Figs. 3D,E). Concurrently, this group showed the weakest positive PAS staining reaction, signifying a profound impairment of colloid synthesis and storage (Fig. 4D).

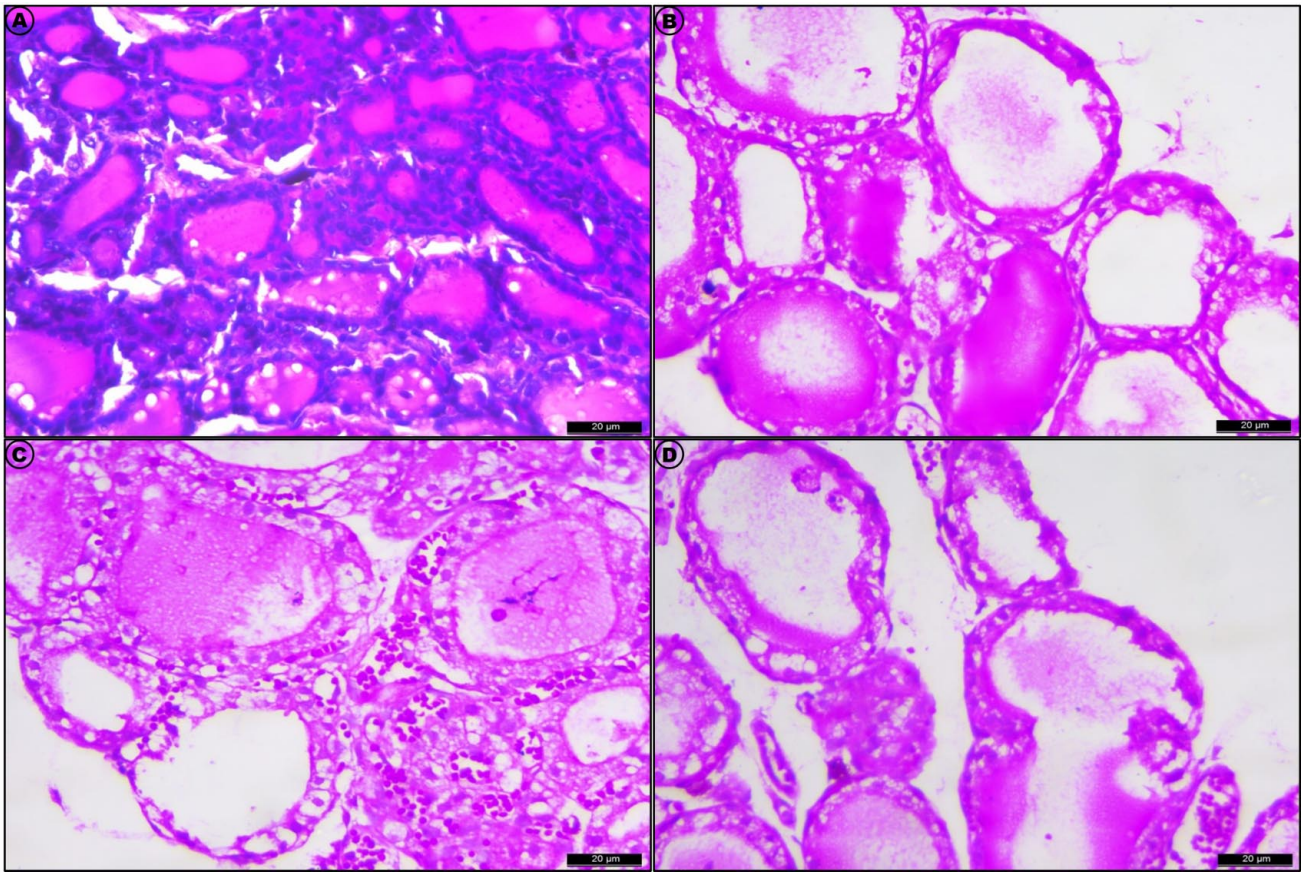

Fig. 4. Periodic Acid Schiff (PAS) staining for colloid glycoprotein content (x400): **A.** A photomicrograph of group I shows a strong positive PAS staining reaction. **B.** A photomicrograph of group II demonstrates a moderate positive PAS staining reaction. **C.** A photomicrograph of group III reveals a mild positive PAS staining reaction. **D.** A photomicrograph of group IV demonstrates a weak positive PAS staining reaction.

Table III. Semi-quantitative scoring of histopathological changes.

Pathological parameter	Group I	Group II	Group III	Group IV
Vascular congestion	Absent/Minimal	Mild	Moderate	Severe
Colloid reduction/Empty lumen	Normal/Full	Mild Reduction	Moderate Reduction	Severe Reduction/Empty lumen
Epithelial exfoliation	Absent	Absent/Rare	Moderate	Pronounced
Inter-sample consistency (rats affected)	0/10	4/10	8/10	10/10

Semi-Quantitative Scoring and Consistency of histological alterations revealed a dose-dependent increase in pathological severity across the semaglutide-treated groups for all three parameters (colloid depletion, vascular congestion, and epithelial exfoliation) compared with the control group. These changes were most pronounced in the high-dose group, indicating progressive structural disruption of thyroid follicles (Table III). Crucially, in the High-Dose Semaglutide Group:

- Inter-sample consistency was high, with all 10 out of 10 samples exhibiting severe vascular congestion.
- Intra-sample consistency was pronounced, with more than 80 % of the examined follicles within individual samples

showing either colloid reduction, an empty lumen, or exfoliated epithelium, directly supporting the visual evidence of widespread deterioration.

2. Quantitative Histological Analysis of follicle diameter (μm): The morphometrical measurements of the follicle diameter showed a non-significant difference in the Low-Dose Semaglutide Group (P = 0.13) but a highly significant decline (P < 0.001) in both the Medium-Dose Semaglutide Group and High-Dose Semaglutide Group when compared to the Control Group, indicating follicular atrophy at higher doses (Table IV, Fig. 3F).

3. IHC Analysis (Ki67, Calcitonin, and BAX). Control Group illustrated negative Ki67 immune-expression (Fig. 5A), a few calcitonin immunoreactive C- cells forming a part of the follicles lining cells and between the follicles (Fig. 6A). Additionally, a negative BAX immune-expression was demonstrated (Fig. 7A)

The Low-Dose Semaglutide Group showed a weak Ki67 positive immunoreaction in the nuclei of few follicular cells (Fig. 5B), a mild positive calcitonin immunoreactive C- cells (Fig. 6B), and a mild positive nuclear and cytoplasmic reaction for Bax (Fig. 7B).

Table IV. Diameter of the follicles in μm among the study groups.

	Group I (N = 10)	Group II (N = 10)	Group III (N = 10)	Group IV (N = 10)	Test	P value
Diameter of the follicles					1.52	0.13[1]
Mean ±SD	209.5±6.3	203.6±8.80	196.3±4.0	187.0±7.2	3.56	<0.001[2]
Range	200 – 220	190 -217	191 – 204	180- 197	3.78	<0.001[3]

U = Mann Whitney U test. 1: Comparing group I and group II. 2: Comparing group I and group III. 3: Comparing group I and group IV.

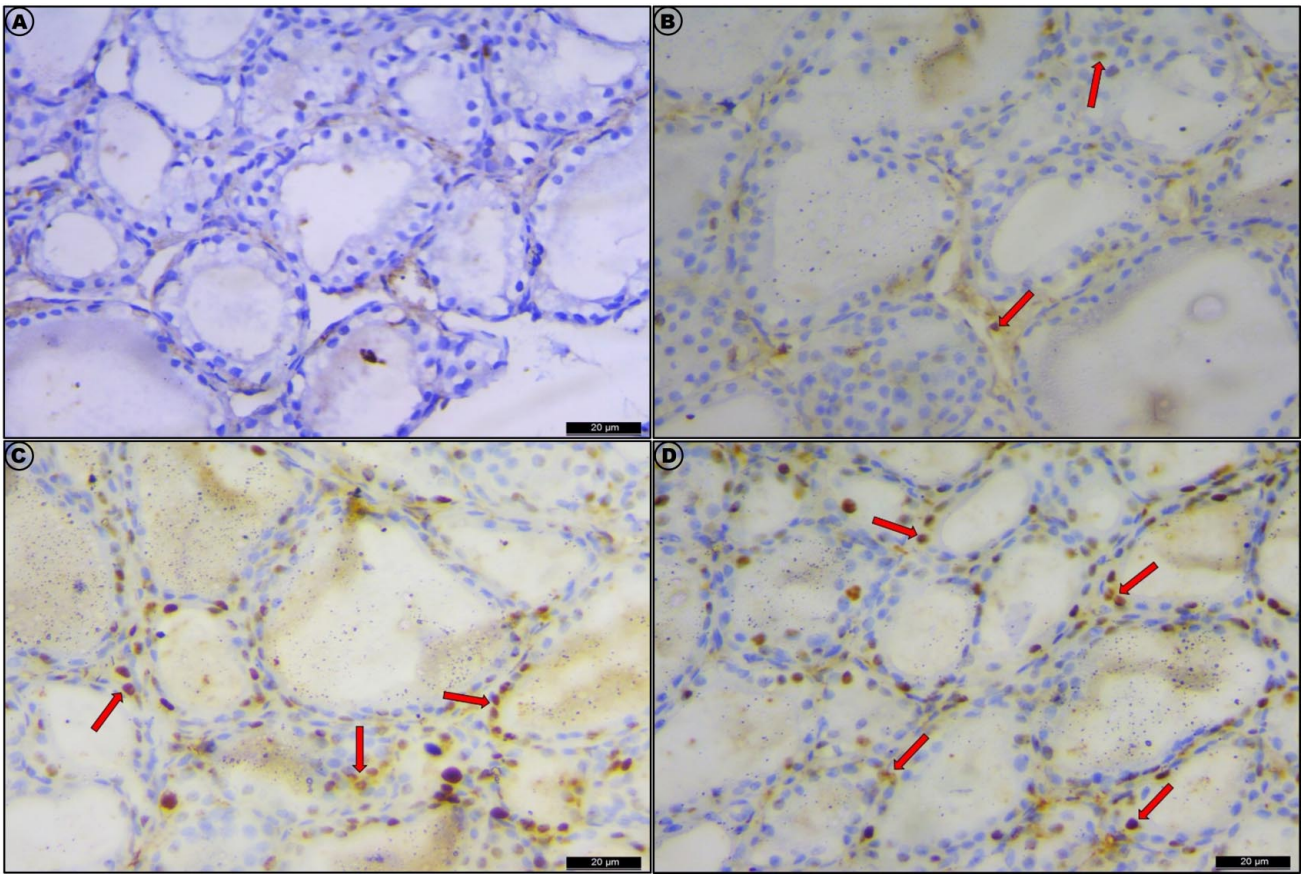

Fig. 5. Immunohistochemical staining for proliferation marker Ki67 (x400). **A.** A photomicrograph of group I reveals a negative Ki67 immune staining reaction. **B**. A photomicrograph of group II demonstrates a weak Ki67 positive immunoreaction in the nuclei of few follicular cells (arrows). **C.** A photomicrograph of group III shows a moderate Ki67 positive immunoreaction in the nuclei of few follicular cells (arrows). **D.** A photomicrograph of group IV demonstrates a strong Ki67-positive immunoreaction in numerous follicular nuclei in multiple follicles (arrows).

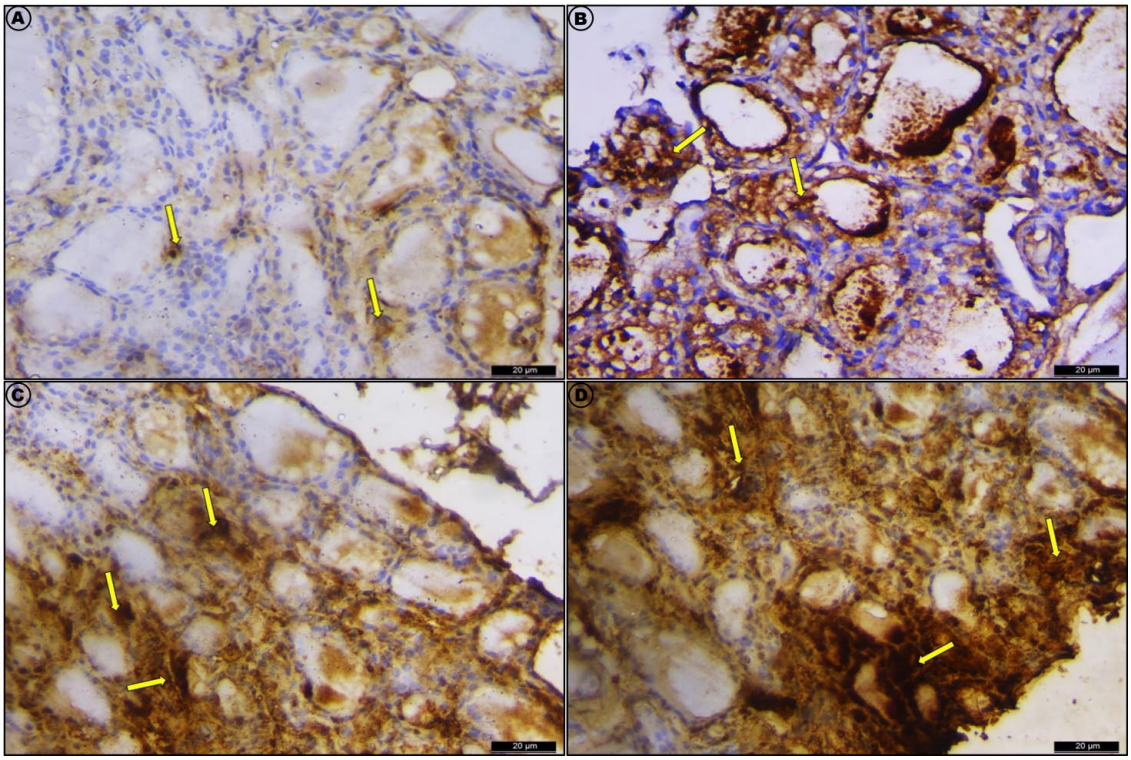

Fig. 6. Immunohistochemical staining for Calcitonin (C-Cell marker) (x400). **A.** A photomicrograph of group I shows a few calcitonin immunoreactive C- cells forming a part of the follicles lining cells and between the follicles (arrows). **B.** A photomicrograph of group II displays a mild positive calcitonin immunoreactive C- cells forming a part of the follicles lining cells and between the follicles (arrows). **C.** A photomicrograph of group III reveals a moderate positive calcitonin immunoreactive C- cells forming a part of the lining cells of the follicles and between the follicles (arrows). **D.** A photomicrograph of group IV shows numerous C- cells forming a part of the follicles lining cells and in between the follicles with intense positive brown calcitonin immune reaction (arrows).

In contrast, a highly significant rise ($P < 0.001$) in the % area of all three positive markers—Ki67 (proliferation), Calcitonin (C-cell mass), and BAX (apoptosis)—was observed in both the Medium-Dose Semaglutide Group (Figs. 5C, 6C and 7C) and the High-Dose Semaglutide Group when compared to the Control Group. This demonstrates a dose-dependent increase in C-cell number and a heightened state of cellular turnover/stress in the follicular epithelium. (Figs. 5D, 6D and 7D).

IHC quantitative image analysis of the area % of positive immunoreactive cells demonstrated a marked, dose-related increase in the immunoreactive area percentage for Ki67 and BAX, indicating enhanced proliferative and apoptotic activity, respectively, in semaglutide-treated thyroid tissue. Similarly, the calcitonin-positive area percentage increased significantly in the medium- and high-dose groups (Table V, Fig. 8).

Table V. Immunohistochemical analysis among the study groups.

	Group I (N = 10)	**Group II** (N = 10)	**Group III** (N = 10)	**Group IV** (N = 10)	**Test**	**P value**
Area % of Ki67					1.02	0.31[1]
Mean ±SD	2.2±0.92	2.8±1.4	11.9±2.92	20.5±3.78	3.82	<0.001[2]
Range	1 – 4	1 – 5	5 – 15	14 – 25	4.11	<0.001[3]
Area % of Calcitonin					1.26	0.21[1]
Mean ±SD	6.9±1.29	7.7±1.16	40.0±7.4	59.6±10.06	3.80	<0.001[2]
Range	5 – 9	6 – 10	22 – 46	41 – 68	4.22	<0.001[3]
Area % of BAX					1.67	0.10[1]
Mean ±SD	2.3±1.06	3.3±1.25	31.9±8.97	59.8±9.79	3.81	<0.001[2]
Range	1 – 4	2 – 5	12 – 40	41 – 68	4.25	<0.001[3]

U = Mann Whitney U test. 1: Comparing group I and group II. 2: Comparing group I and group III. 3: Comparing group I and group IV.

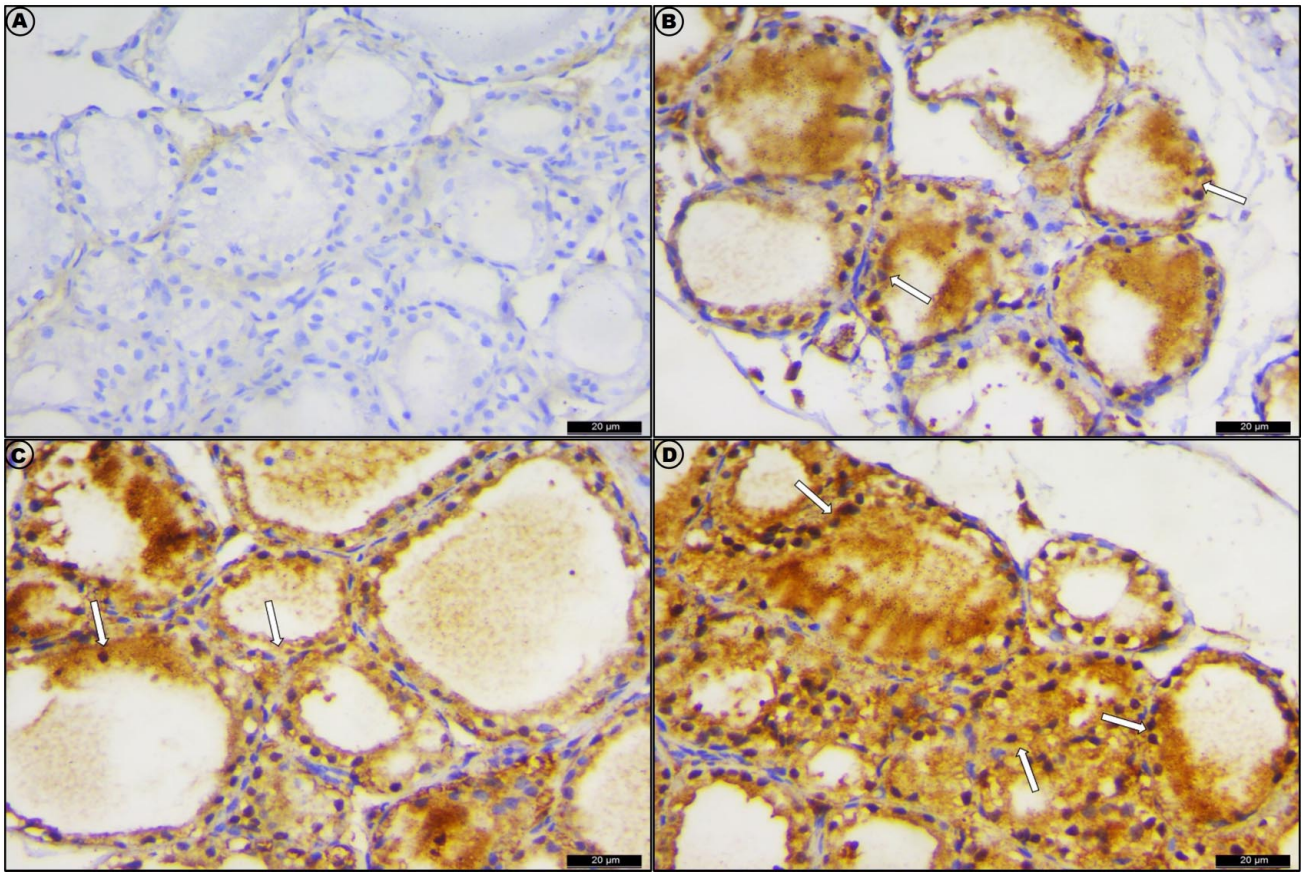

Fig. 7. Immunohistochemical staining for apoptotic marker BAX (x400). **A.** A photomicrograph of group I reveals a negative BAX expression in the cytoplasm and nuclei of all follicular lining epithelium. **B.** A photomicrograph of group II demonstrates a mild positive cytoplasmic and nuclear reaction for BAX (arrows). **C.** A photomicrograph of group III shows a moderate positive cytoplasmic and nuclear reaction for BAX (arrows). **D.** A photomicrograph of group IV demonstrates a strong positive cytoplasmic and nuclear reaction for BAX (arrows).

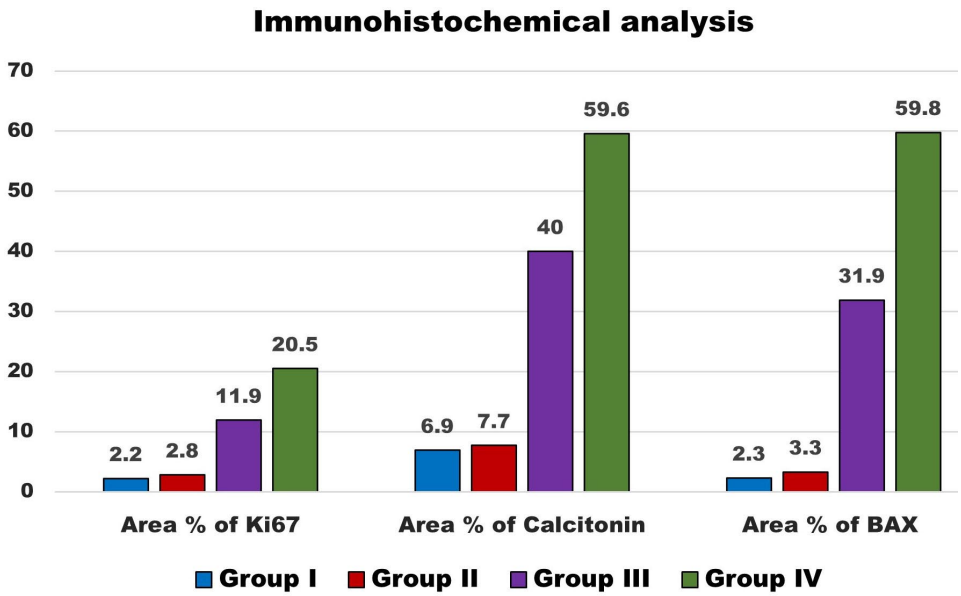

Fig. 8. Quantitative mean area % of positive Ki67, Calcitonin, and BAX Immunoreactivity in the study groups

DISCUSSION

The potential therapeutic role of oral semaglutide in the clinical management of both obesity and diabetes mellitus has been accepted by the U.S. Food and Drug Administration (Ibrahim *et al*., 2024).

Our investigation confirms this pharmacological profile, as the body weight of the treated male albino rats decreased in a pronounced dose-dependent manner. These findings are consistent with previous research by Amaro *et al*. (2022), who reported that clinical studies in individuals with obesity demonstrate that semaglutide promotes weight loss through multiple biological mechanisms that collectively reduce overall energy intake. Similarly, Rakhat *et al*. (2023), mentioned that oral semaglutide gradually reduces food intake with a notable reduction of body weight gain during a 3-day treatment period in mice, corroborating the central appetite regulation effect.

In the current study, hormonal assays for thyroid function revealed that the Low-Dose Semaglutide Group showed a minimal decrease in T3, T4, and TSH, while a more notable and highly significant decline was observed in the Medium-Dose and High-Dose Groups when compared with the Control Group. This observed decline may be attributed to the drug's structural deteriorating effect on the thyroid follicles, which was confirmed in the histological results.

Scientific literature concerning the mechanism by which GLP-1 receptor activation affects thyroid function is often conflicting and limited (Capuccio *et al*., 2024). While the precise process is still being determined, several studies indicate that GLP-1 receptor activation may affect thyroid hormone levels and, more generally, thyroid function parameters.

Sencar *et al*. (2019) examined 46 diabetic individuals receiving exenatide and reported that serum TSH levels significantly decreased without a significant alteration in free T4, T3, or calcitonin levels before and after treatment.

Similarly, Ye *et al*. (2022) demonstrated that liraglutide treatment decreased TSH levels in a group of 49 diabetic individuals. Crucially, this TSH reduction has been linked to metabolic improvement; Tee *et al*. (2023) discovered that obese individuals with type 2 diabetes who lost 6.5 % of their body weight after receiving exenatide for a year had a significant but slight drop in mean TSH levels, but no change in free T4 levels. Their data further showed no alterations in TSH levels in those who did not lose weight while on exenatide therapy (Sencar *et al*., 2019). Our study's finding of a concurrent, highly significant reduction in T3 and T4 levels alongside TSH in the rat model suggests that the effect of oral semaglutide is likely more pervasive than purely a weight-loss mediated TSH suppression, involving a direct impact on the gland's synthetic capacity.

Histological sections of the Low-Dose Semaglutide Group showed mild changes including congested blood vessels and some follicles with a decrease in colloidal material, while others appeared with an empty lumen. The Medium-Dose Group revealed more deterioration, with greater reduction in colloidal material. The High-Dose Group showed the most severe deterioration, revealing exfoliated follicular epithelium, follicular vacuolation, and severe vascular congestion. This structural decline corresponded with the morphometric data: the diameter of the follicles showed a non-significant difference in the Low-Dose Group ($P = 0.13$) but a highly significant decline in both the Medium-Dose Group and High Dose Group ($P < 0.001$) when compared to the Control Group. This matches prior animal model studies which demonstrated that the use of GLP-1 receptor agonists, such as liraglutide and exenatide, causes abnormal alterations in thyroid C cells (Martín-Lacave *et al*., 2009). Moreover, prescribing guidelines for semaglutide and other FDA-approved long-acting GLP-1RAs indicate that these agents have been associated with thyroid C-cell tumors, including medullary thyroid carcinoma, in animal studies (Amaro *et al*., 2022). However, Capuccio *et al*. (2024) reported that liraglutide and exenatide lead to the gradual development of thyroid hyperplasia and adenomas. Furthermore, the dose-dependent reduction in PAS staining intensity, which was weakest in the High-Dose Group, aligns with the findings of Mandour *et al*. (2022) that a decline in colloid immunohistochemical analyses could impair the functional integrity of the thyroid gland and subsequently hinder the biosynthesis of thyroid hormones.

Regarding the immunohistochemical analyses, the results provide evidence of dual cellular responses.

First; C-Cell Hyperplasia (On-Target Effect), Calcitonin-stained sections showed a progression from a few immunoreactive C-cells in the Control Group to a strong positive brown calcitonin immunoreaction in numerous C-cells forming part of the follicular lining and between follicles in the High-Dose Group. Madsen *et al*. (2012) declared that prolonged GLP-1 receptor agonist activation is linked to elevated calcitonin levels and tumor development in rats and mice. After investigating potential explanations, they discovered a definite GLP-1 receptor agonist dependence of the C-cell stimulatory effects, namely elevated calcitonin and C-cell hyperplasia. Rosol (2013) further reported that GLP-1 receptor agonists have been

demonstrated to promote C-cell hyperplasia and neoplasia in both sexes of rats and mice, noting that rat C-cells are more susceptible than those of mice to these effects.

Second; Follicular Cell Stress and Apoptosis (Novel Finding), Ki67-stained sections, which were negative in the Control Group, progressed from a weak positive immunoreaction in the Low-Dose Group to a strong positive immunoreaction in numerous follicular nuclei in the High-Dose Group. Concurrently, BAX-stained sections progressed from negative expression in the Control Group to a strong positive nuclear and cytoplasmic reaction in the High-Dose Group. These findings suggest a state of high cellular turnover and stress within the follicular epithelium. While Wang *et al*. (2025) reported that semaglutide significantly decreased Ki67 expressions and encouraged apoptosis in oral squamous cell carcinoma cells through the P38 MAPK signaling pathway, our finding in the thyroid follicular cells suggests a compensatory proliferative drive (Ki67) against drug-induced toxicity, which is ultimately overcome by increased BAX-mediated apoptosis (Madsen *et al*., 2012). This follicular degeneration, distinct from the C-cell proliferation, is the primary source of the T3/T4 synthesis impairment.

The observed hormonal suppression and histological alterations following oral semaglutide administration may be explained by several potential mechanisms. First, semaglutide, like other GLP-1 receptor agonists, may exert direct effects on thyroid parafollicular (C) cells, which express functional GLP-1 receptors capable of modulating calcitonin secretion and local cellular activity. Second, indirect systemic mechanisms could contribute: GLP-1RA-induced changes in glucose metabolism, insulin sensitivity, and energy homeostasis may influence hypothalamic–pituitary–thyroid axis regulation, thereby reducing circulating T3, T4, and TSH levels. Third, the concurrent upregulation of Ki67 and BAX immunoreactivity suggests a disturbed proliferative–apoptotic balance within the thyroid parenchyma, which may reflect adaptive or stress-related cellular turnover rather than neoplastic transformation. These proposed pathways are consistent with recent reports describing species- and dose-dependent thyroid responses to GLP-1RAs in rodents and humans, underscoring the importance of further mechanistic studies to delineate receptor distribution and intracellular signaling cascades in thyroid tissue.

CONCLUSION

Oral semaglutide exhibits notable detrimental effects on the rat thyroid gland parameters in a dose-dependent manner. The study moves beyond descriptive confirmation by elucidating a dual cellular mechanism: a direct, expected C-cell proliferative effect alongside a novel, indirect follicular cell degenerative effect marked by heightened BAX-mediated apoptosis and failed regeneration. Given the potential for thyroid C-cell cancers in animals, these findings necessitate careful consideration regarding the clinical use of this drug in human populations and warrant further research to elucidate the underlying cellular and molecular pathways and translational relevance.

SALAMA, R. M.; ESSAWY, A. S. & OMAR, M. A. Efectos notables del agonista del receptor del péptido similar al glucagón-1 en la glándula tiroides de ratas albinas macho. *Int. J. Morphol., 44(2)*:542-554, 2026.

RESUMEN: Se han observado efectos de los agonistas del receptor del péptido similar al glucagón-1 en la glándula tiroides, particularmente en las células C parafoliculares, con formulaciones inyectables, pero la información completa sobre la nueva formulación oral de semaglutida sigue siendo limitada. El objetivo del estudio fue investigar y cuantificar los efectos dependientes de la dosis de la administración oral crónica de semaglutida sobre la función endocrina, la morfología y los marcadores celulares de proliferación y apoptosis en la glándula tiroides de ratas albinas macho. Cuarenta (40) ratas macho adultas se dividieron en 4 grupos; Un grupo control y tres grupos de tratamiento con semaglutida (dosis baja, dosis media y dosis alta), que corresponden al rango terapéutico humano de 3 a 14 mg, fueron tratados durante 30 días. El estado endocrino se evaluó mediante los niveles séricos de triyodotironina (T3), tiroxina (T4) y hormona estimulante de la tiroides (TSH). El análisis histológico incluyó tinción con hematoxilina y eosina (Hx&E), reacción de ácido peryódico de Schiff (PAS) y cuantificación inmunohistoquímica (IHC) de Ki67, calcitonina y BAX. Todos los grupos tratados mostraron una reducción significativa y dependiente de la dosis en el peso corporal. Los grupos de dosis media y alta mostraron una disminución altamente significativa (P<0,001) en las 3 hormonas tiroideas séricas medidas. El examen patológico reveló un deterioro proporcional a la dosis, incluyendo atrofia folicular y exfoliación epitelial. La inmunohistoquímica confirmó un aumento altamente significativo y dependiente de la dosis en el número de células C inmunorreactivas a la calcitonina y la expresión de Ki67 y BAX en los grupos de dosis más altas (P<0,001). La semaglutida oral induce efectos perjudiciales notables y dependientes de la dosis en la integridad morfológica y endocrina de la glándula tiroides de la rata, particularmente en dosis equivalentes a 7 y 14 mg en humanos. Esto exige mayor investigación para dilucidar los mecanismos moleculares subyacentes.

PALABRAS CLAVE: Semaglutida; TSH; Ki67; Calcitonina; BAX.

REFERENCES

Amaro, A.; Sugimoto, D. & Wharton, S. Efficacy and safety of semaglutide for weight management: evidence from the STEP program, Postgrad. *Med., 134(sup.1)*:5-17, 2022.

Andersen, A.; Knop, F. K. & Vilsbøll, T. A. Pharmacological and Clinical Overview of Oral Semaglutide for the Treatment of Type 2 Diabetes. *Drugs, 81(9)*:1003-30, 2021.

Anderson, S. L.; Beutel, T. R. & Trujillo, J. M. Oral semaglutide in type 2 diabetes. *J. Diabetes Complications, 34(4)*:107520, 2020.

Barnett, A. H. The role of GLP-1 mimetics and basal insulin analogues in type 2 diabetes mellitus: Guidance from studies of liraglutide. *Diabetes Obes. Metab., 14(4)*:304-14, 2012.

Campbell, J. E. & Drucker, D. J. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab., 17(6)*:819-37, 2013.

Capuccio, S.; Scilletta, S.; La Rocca, F.; Miano, N.; Di Marco, M.; Bosco, G.; Di Giacomo Barbagallo, F.; Scicali, R.; Piro, S. & Di Pino, A. Implications of GLP-1 receptor agonist on thyroid function: a literature review of its effects on thyroid volume, risk of cancer, functionality and TSH levels. *Biomolecules, 14(6)*:687, 2024.

Choe, H. J. & Cho, Y. M. Peptidyl and non-peptidyl oral glucagon-like peptide-1 receptor agonists. *Endocrinol. Metab., 36(1)*:22-9, 2021.

Flint, A.; Kapitza, C.; Hindsberger, C. & Zdravkovic, M. The once daily human glucagon-like peptide-1 (GLP-1) analog liraglutide improves postprandial glucose levels in type 2 diabetes patients. *Adv. Ther., 28(3)*:213-26, 2011.

Gibbons, C.; Blundell, J.; Hoff, S. T.; Dahl, K.; Bauer, R. & Bækdal, T. Effects of oral semaglutide on energy intake, food preference, appetite, control of eating and body weight in subjects with type 2 diabetes. *Diabetes Obes. Metab., 23(2)*:581-8, 2021.

Gogineni, P.; Melson, E.; Papamargaritis, D. & Davies, M. Oral glucagon-like peptide-1 receptor agonists and combinations of entero-pancreatic hormones as treatments for adults with type 2 diabetes: where are we now? *Expert Opin. Pharmacother., 25(7)*:801-18, 2024.

Hadie, S.; Abdul Manan, H. & Abdulla, S. Thyroid gland resection in euthanized rat. A practical guide. *Int. Med. J., 20(99)*:102, 2013.

Husain, M.; Birkenfeld, A. L.; Donsmark, M.; Dungan, K.; Eliaschewitz, F. G.; Franco, D. R.; Jeppesen, O. K.; Lingvay, I.; Mosenzon, O.; Pedersen, S. D.; *et al*. Oral semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N. Engl. J. Med., 381(9)*:841-51, 2019.

Ibrahim, S. S.; Ibrahim, R. S.; Arabi, B.; Brockmueller, A.; Shakibaei, M. & Büsselberg, D. The effect of GLP-1R agonists on the medical triad of obesity, diabetes, and cancer. *Cancer Metastasis Rev., 43(4)*:1297-314, 2024.

Knudsen, L. B.; Madsen, L. W.; Andersen, S.; Almholt, K.; Boer, A. S.; Drucker, D. J.; Gotfredsen, C.; Egerod, F. L.; Hegelund, A. C.; Jacobsen, H.; *et al*. Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. *Endocrinology, 151(4)*:1473-86, 2010

Köseog˘lu, D.; Bas¸er, Ö.; Berker, D. & Güler, S. Exenatide treatment for 6 months decreased serum TSH levels and thyroid volume, but had no effect on thyroid nodules and serum CEA and calcitonin levels. *Acta Endocrinol. (Buchar), 16(3)*:275-9, 2020.

Lisco, G.; De Tullio, A.; Disoteo, O.; Piazzolla, G.; Guastamacchia, E.; Sabbà, C.; De Geronimo, V.; Papini, E. & Triggiani, V. Glucagon-like peptide 1 receptor agonists and thyroid cancer: is it the time to be concerned?. *Endocr. Connect., 12(11)*:e230257, 2023.

Madsen, L. W.; Knauf, J. A.; Gotfredsen, C.; Pilling, A.; Sjögren, I.; Andersen, S.; Andersen, L.; Boer, A. S.; Manova, K.; Barlas, A.; *et al*. GLP-1 receptor agonists and the thyroid: C-cell effects in mice are mediated via the GLP-1 receptor and not associated with RET activation. *Endocrinology, 153(3)*:1538-47, 2012.

Mandour, D. A.; Abdelfattah, M. T.; Shuaib, D. M. & Sabry, R. Cytoarchitecture changes of the rat thyroid gland following furan administration implemented fas-apoptotic pathway with potential abrogating role of selenium. *Zagagig Univ. Med. J., 28(4)*:871-82, 2022.

Martín-Lacave, I.; Borrero, M. J.; Utrilla, J. C.; Fernández-Santos, J. M.; Miguel, M.; Morillo, J.; Guerrero, J. M.; García-Marín, R. & Conde, E. C cells evolve at the same rhythm as follicular cells when thyroidal status changes in rats. *J. Anat., 214(3)*:301-9, 2009.

Matsuu-Matsuyama, M.; Shichijo, K.; Okaichi, K.; Kurashige, T.; Kondo, H.; Miura, S. & Nakashima, M. Effect of age on the sensitivity of the rat thyroid gland to ionizing radiation. *J. Radiat. Res., 56(3)*:493-501, 2015.

Pratley, R.; Amod, A.; Hoff, S. T.; Kadowaki, T.; Lingvay, I.; Nauck, M.; Nauck, M.; Pedersen, K. B.; Saugstrup, T. & Meier, J. J. Oral semaglutide versus subcutaneous liraglutide and placebo in type 2 diabetes (PIONEER 4): A randomised, double-blind, phase 3A trial. *Lancet, 394(10192)*:39-50, 2019.

Pyke, C.; Heller, R. S.; Kirk, R. K.; Ørskov, C.; Reedtz-Runge, S.; Kaastrup, P.; Hvelplund, A.; Bardram, L.; Calatayud, D. & Knudsen, L. B. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology, 155(4)*:1280-90, 2014.

Rakhat, Y.; Wang, L.; Han, W.; Rustemova, A.; Kulzhanova, N.; Yamada, Y.; Yabe, D.; Seino, Y. & Yada, T. Oral semaglutide under human protocols and doses regulates food intake, body weight, and glycemia in diet-induced obese mice. *Nutrients, 15(17)*:3765, 2023.

Rosol, T. J. On-target effects of GLP-1 receptor agonists on thyroid C-cells in rats and mice. *Toxicol Pathol., 41(2)*:303-9, 2013.

Ruska, Y.; Peterfi, Z.; Szilvásy-Szabó, A.; Kóvári, D.; Hrabovszky, E.; Dorogházi, B.; Gereben, B.; Tóth, B.; Matziari, M.; Wittmann, G.; *et al*. GLP-1 receptor signaling has different effects on the perikarya and axons of the hypophysiotropic thyrotropin-releasing hormone synthesizing neurons in male mice. *Thyroid, 34(2)*:252-60, 2024.

Sencar, M. E.; Sakiz, D.; Calapkulu, M.; Hepsen, S.; Kizilgul, M.; Ozturk, I. U.; Ucan, B.; Bayram, M.; Cagir, B. B.; Akin, S.; *et al*. The effect of exenatide on thyroid-stimulating hormone and thyroid volume. *Eur. Thyroid J., 8(6)*:307-11, 2019.

Tee, S. A.; Tsatlidis, V. & Razvi, S. The GLP-1 Receptor Agonist Exenatide Reduces Serum TSH by Its Effect on Body Weight in People with Type 2 Diabetes. *Clin. Endocrinol., 99(4)*:401-8, 2023.

Van Noorden, S. & Polak, M. *Immunocytochemistry: Practical Applications in Pathology and Biology*. 2nd ed. Edinburgh, Churchill Livingstone, 2014. pp.31-40.

Wang, C.; Wu, Z.; Zhou, J.; Cheng, B. & Huang, Y. Semaglutide, a glucagon-like peptide-1 receptor agonist, inhibits oral squamous cell carcinoma growth through P38 MAPK signaling pathway. *J. Cancer Res. Clin. Oncol., 151(3)*:103, 2025.

Yabe, D.; Nakamura, J.; Kaneto, H.; Deenadayalan, S.; Navarria, A.; Gislum, M.; Inagaki, N. & PIONEER 10 Investigators. Safety and efficacy of oral semaglutide versus dulaglutide in Japanese patients with type 2 diabetes (PIONEER 10): an open-label, randomised, active-controlled, phase 3a trial. *Lancet Diabetes Endocr., 8(5)*:392-406, 2020.

Ye, J.; Xu, J.; Wen, W. & Huang, B. Effect of Liraglutide on Serum TSH Levels in Patients with NAFLD and Its Underlying Mechanisms. *Int. J. Clin. Pract., 2022*:1786559, 2022.

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