

Beneficial Effects of *Zingiber officinale* Rhizome Powder on Thyroid and Liver of Male Rabbit Fed a High Fat Diet

Efectos Beneficiosos del Polvo de Rizoma de *Zingiber officinale* Sobre la Tiroides y el Hígado de Conejos Machos Alimentados con una Dieta Rica en Grasas

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SUMMARY: Palm oil, rich in fatty acids, is extensively used in the food industry. Excessive intake of high-fat diets (HFD) can lead to ectopic lipid accumulation, adversely affecting tissue structure and function, particularly in the liver and thyroid. Ginger (*Zingiber officinale*) possesses various biological properties, including antioxidant, anti-inflammatory, and hypolipidemic effects. This study aimed to investigate whether ginger powder exerts protective effects against lipid and hormonal disturbances induced by a HFD and its impact on cellular architecture of the thyroid and liver in male rabbits. Total phenolic and flavonoid contents were quantified, and phenolic compounds were identified via high-performance liquid chromatography (HPLC). Hormonal levels were measured using appropriate assays. Histological examinations of the thyroid and liver tissues were performed, with immunohistochemistry applied specifically to thyroid tissue to assess cellular changes. Ginger supplementation demonstrated protective effects by improving lipid and hormonal profiles and restoring cellular architecture in both tissues. Histologically, cells exhibited reduced cytoplasmic vacuolation, and there was a re-establishment of blood capillaries, along with the disappearance of hemorrhages. The high vitamin C content in ginger likely contributed to its antioxidant activity, protecting tissues from oxidative stress. The phenolic compounds, including rutin, kaempferol, and caffeic acid, appeared to mediate these effects by decreasing fibrosis and inflammation. These findings suggest that ginger has significant protective effects against HFD-induced tissue alterations, primarily through its antioxidant properties and phenolic constituents. The results provide insights into the mechanisms by which phenolic compounds mitigate fibrosis and inflammation. Future research should explore the therapeutic potential of specific compounds such as rutin, kaempferol, vitamin C, and caffeic acid for protecting thyroid and liver tissues under metabolic stress conditions.

KEY WORDS : *Zingiber officinale* / phenolic compounds; Thyroid function ; Liver function; Lipid metabolism; Free thyroxine-cortisol.

INTRODUCTION

In humans and animals, a high-fat diet (HFD) induces obesity, which disrupts endocrine systems (Apovian *et al.*, 2015), including the thyroid (Camastra *et al.*, 2009), adrenal glands (Auvinen *et al.*, 2012), pancreas, and adipose tissue, leading to chronic release of insulin and leptin (Chakraborty *et al.*, 2016). Furthermore, this condition damages tissues and organs, affecting the morphology and metabolism of the liver (Bravo *et al.*, 2011).

The anterior pituitary secretes thyrotropin-stimulating hormone (TSH) into the bloodstream in response to feedback from free thyroxine (FT4) and free triiodothyronine (FT3). Thyroid transcription factor-1 (TTF-1) is expressed in the

nuclei of thyrocytes and is a crucial protein involved in the synthesis of thyroid hormones.

Thyroid hormones play a vital role in regulating lipid metabolism, closely modulating hepatic lipid processes, including de novo lipogenesis, triglyceride (TG) accumulation, lipolysis, fatty acid oxidation, and the biosynthesis and clearance of cholesterol (Sinha *et al.*, 2018). Numerous studies have shown a negative correlation between free T4 levels and total cholesterol and LDL cholesterol in euthyroid subjects (Roos *et al.*, 2007).

Activation of the hypothalamic-pituitary-adrenal

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(HPA) axis begins in the hypothalamus, where corticotropin-releasing hormone (CRH) is released. CRH stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH), which then stimulates the zona fasciculata of the adrenal cortex to synthesize and release cortisol in humans and corticosterone in rodents (Kyrou & Tsigos, 2009).

Natural compounds derived from plants, such as spices, have demonstrated notable health benefits. Currently, ginger rhizome is used globally as a spice, and its extracts exhibit pharmacological activities including hypoglycemic, insulinotropic, and hypolipidemic effects in humans (Huang *et al.*, 2004) and animal models. The antioxidant compounds in ginger include gingerols, shogaols, monoterpenes, sesquiterpenes, and various phenolic compounds.

This study aims to investigate the phenolic compounds of ginger using high-performance liquid chromatography (HPLC), alongside lipid and hormonal levels (free T4, cortisol). Correlations between hormone and lipid levels, histology of the thyroid and liver, and immunohistochemical analysis of the thyroid will be performed to assess the efficacy of selected ginger powder in male rabbits fed an HFD.

MATERIAL AND METHOD

Plant Material and Preparation of Aqueous Extract.

Dried rhizomes of *Zingiber officinale* were purchased from local arborists in Algiers, Algeria, in June 2021, and ground into fine particles after drying. 10 g of dried powdered material was placed in 100 mL of distilled water, and the mixture was macerated for 30 min at room temperature under magnetic stirring. The supernatant was separated by centrifugation at 3000 rpm for 15 min. The operation was repeated three times with the same plant material to maximize the extraction of secondary metabolites. After each centrifugation, the mixture was filtered through Wattman paper. The filtrate was collected in a numbered 50 mL flask and stored at 4 °C until use. The extraction method followed the procedure described by Boursier *et al.* (2011).

Determination of Total Polyphenolics Content. Total Polyphenolics Content (TPC) was determined using the Folin-Ciocalteu method (Williamson & Carughi, 2011). A volume of 200 µL of the plant extract was mixed with 1 mL of Folin-Ciocalteu reagent (diluted 10 times, Code: 0387000500, Batch: LM03732106) and incubated at room temperature for 5 min. Subsequently, 800 µL of 7.5 % sodium carbonate (Na₂CO₃) solution was added. The absorbance was measured at 765 nm using a UV-Visible spectrophotometer after an incubation period of 2 h at room temperature. Results were expressed as Gallic Acid Equivalents (GAE, milligrams) per 100 g of dried extract.

Determination of Total Flavonoids Content. Total Flavonoids Content (TFC) was assessed via the aluminum chloride (AlCl₃) colorimetric method (Williamson & Carughi, 2011). 1 ml of the extract t was mixed with 1 mL of 2 % aluminum trichloride in methanol. After 10 min of incubation at room temperature, absorbance was measured at 430 nm with an ultraviolet (UV) Visible spectrophotometer. Flavonoids were quantified as milligrams of quercetin equivalents (QE) per 100 g of dry weight.

Flavonoid Extract Preparation and Analysis of Phenolic Components. Aliquots of rhizomes (0.25 g) were extracted with 60 % aqueous methanol (20 mL). Subsequently, 5 mL of 6 M HCl was added to produce a 25 mL solution with 1.2 M HCl in 50 % aqueous methanol. The extracts were refluxed at 90 °C for 2 h. Aliquots of 500 µL, taken before and after hydrolysis, were filtered through a 0.45 µm filter (Crozier *et al.*, 1997).

Quantification of phenolic compounds was performed using High-Performance Liquid Chromatography (HPLC). The HPLC system (German brand, Agilent) included a Model 1100 pump. Separation was achieved on a C18 column (5 µm, 4.6 × 250 mm, Knauer) at 30 °C, with a UV-Visible detector. The mobile phase consisted of solvent A (0.1 % acetic acid in ultrapure water) and solvent B (HPLC-grade methanol). The mobile phase was filtered through a 0.45 µm membrane filter before use. The flow rate was 1 mL/min, and detection was at 254 nm. Identification of flavonoids was based on retention times, UV spectra, and UV absorbance ratios, verified by co-injection with standards purchased from Sigma–Aldrich (USA).

Animals. Male rabbits *Oryctolagus cuniculus*, weighing 1600–2070 g and aged approximately 2.5 months, were obtained from the Breeding Technical Institute in Baba Ali, Blida, Algeria. They were housed individually in cages under natural light conditions and fed with standard pellets (Agricultural Supply Service Cooperative, Blida) and water *ad libitum*. Synthetic breed rabbits were housed in a controlled environment and received a balanced diet, with constant access to clean drinking water. They were regularly monitored for signs of stress and overall well-being. All procedures were conducted with care to minimize stress. A veterinarian was consulted to ensure compliance with animal welfare standards.

This study was conducted in accordance with procedures that were carried out in accordance with Algerian legislation (Law 12-235/2012 relating to the protection of animals) and also in accordance with Algerian legislation (Law 12-235/2012 relating to the protection of animals).

High Fat Diet (HFD). Refined palm oil, solid at room temperature with a melting point of 38-40 °C, was used in this study.

Evaluation of Protective Activity on Thyroid Gland and Liver. An aqueous extract of ginger, containing phenolic compounds and exhibiting antioxidant activity, was prepared by mixing 100 g of standard pellets with 5 g of ginger powder in 100 mL of hot distilled water, and then formed into small pellets. This mixture was used to evaluate effects on lipid profile, hormones, and tissue status (thyroid and liver). Methods followed those described by Seo *et al.* (2021).

Twenty-five male “*Oryctolagus cuniculus*” rabbits were divided into three groups:

- Group I (control, n=6): received 100 g of standard diet and water ad libitum for 10 weeks.
- Group II (n=9): received HFD with 100 g standard diet plus 10 g of palm oil for 10 weeks.
- Group III (n=10): received HFD for 7 weeks, then a mixture of 100 g standard diet and 4 g/kg ginger powder for the final 3 weeks.

The thyroid glands and liver were excised, fixed in 10 % paraformaldehyde for histological analysis, with immunohistochemistry performed only on thyroid glands.

Plasma Lipid Levels. Blood samples were collected into heparinized tubes and centrifuged at 3000 rpm for 10 min. Plasma triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol were measured using enzymatic methods (Kaplan & Pesce, 1984; Naito & Kaplan, 1984) with Mindray reagents on a Mindray BS series automated spectrophotometer (Mindray, Shenzhen, China).

Plasma Hormone Levels. Levels of FT4 and cortisol were measured using a competitive electrochemiluminescence immunoassay (Shenzhen New Industries Biomedical Engineering).

Histological Study. Thyroids and livers were dissected and fixed in 10 % paraformaldehyde, dehydrated through an ethanol series, cleared in xylene, embedded in paraffin (Leica Biosystems, Richmond), and sectioned at 2–3 µm thickness. Sections were deparaffinized, rehydrated, and stained with Heidenhain’s Azan (Gabe, 1968).

Follicle size and colloid area were measured using AxioVision 4.6.3 software (Carl Zeiss). The follicular length (FL) was measured between two distant points within the follicle, and the follicular width (FW) was measured perpendicular to FL. Four epithelial cell height

(ECH) measurements per follicle were also recorded. The follicular size (FS) was calculated with the formula:

$$FS = \frac{FL + FW}{2}$$

For each parameter analyzed at 100× magnification, 100 measurements were performed. For each group of rabbits, so on average 20 randomly selected measurements per animal.

Heidenhain Azan Staining. Sections were deparaffinized in xylene and hydrated in distilled water. Nuclear fast red (Sigma-Aldrich) was applied for 5 min, then sections were rinsed. Tungstophosphoric acid (H₃PO₄) was used for 30–60 min for differentiation, followed by rinsing. Sections were then stained with Heidenhain blue (Orange G sodium salt and Aniline blue, Germany), dehydrated in alcohol, cleared in xylene, and mounted in Eukitt (Sigma-Aldrich).

Immunohistochemistry. To assess TTF-1 expression, sections from thyroid tissue of each group were deparaffinized, microwaved at 100 °C for 30 min for antigen retrieval, and blocked with 3 % hydrogen peroxide for 10 min. Sections were incubated overnight at 4 °C with a mouse monoclonal anti-TTF-1 antibody (BMS016, Zytomed). Negative controls used IgG instead of primary antibody. Detection employed a streptavidin-peroxidase kit (Invitrogen), visualized with diaminobenzidine (DAB), and counterstained with hematoxylin. Sections were observed under a light microscope.

Microscopic Analysis. Images were captured with an OPTICA AXIOM 2000 microscope equipped with a high-resolution camera (MA88-500) at various magnifications (×100, ×400, ×1000). Image analysis was performed with TS Viewer and TS Analyzer software (2011.8). Follicle and colloid measurements were carried out using AxioVision 4.6.3 (Carl Zeiss).

Statistical Analysis. Data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software version 8.0.2 (GraphPad Software, Inc.). Differences between groups were assessed using the unpaired Student’s t-test for comparisons between two groups. One-way analysis of variance (ANOVA) followed by Tukey’s post hoc test was used for multiple comparisons. Pearson’s correlation coefficient was calculated using Statistica software version 8.0 (StatSoft, Inc.) to evaluate the relationship between variables. A p-value less than 0.05 was considered statistically significant.

RESULTS

The ginger extract contained 8.3 ± 0.36 mg of gallic acid equivalents per gram of extract for total polyphenols, and the total flavonoid content was equivalent to 4.17 ± 0.2 mg of quercetin per gram (Table I).

HPLC analysis allowed the identification and quantification of phenolic compounds in the aqueous ginger extract. The LC chromatogram is shown in Figure 1 and Table II summarizes the peak numbers, retention times (RT), concentrations, and identified compounds. Identification was confirmed by comparing retention times and ultraviolet (UV) absorbance maxima with authentic standards.

Table I. Total Polyphenols (mg GAE/g) and Flavonoids (mg QE/g) Content in Aqueous Extract of *Zingiber officinale*.

Sample	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
Aqueous extract	8.3 ± 0.36	4.17 ± 0.2

Values are the mean \pm SEM (n=3). Means with different letters were significantly different at $p < 0.05$.

Table II. Contents of Phenolic Acids and Flavonoids Identified from Aqueous Herbal Extract by HPLC.

Peak	Time retentions	% of Compound	Compound
1	3,055	34,250	Ascorbicacid
2	6,350	2,240	Gallicacid
3	7,033	2,560	Tannicacid
4	19,102	1,043	Cafeicacid
5	28,633	1,350	indol-3-carboxylicacid
6	30,621	2,810	Rutin
7	41,672	2,545	Kaempferol

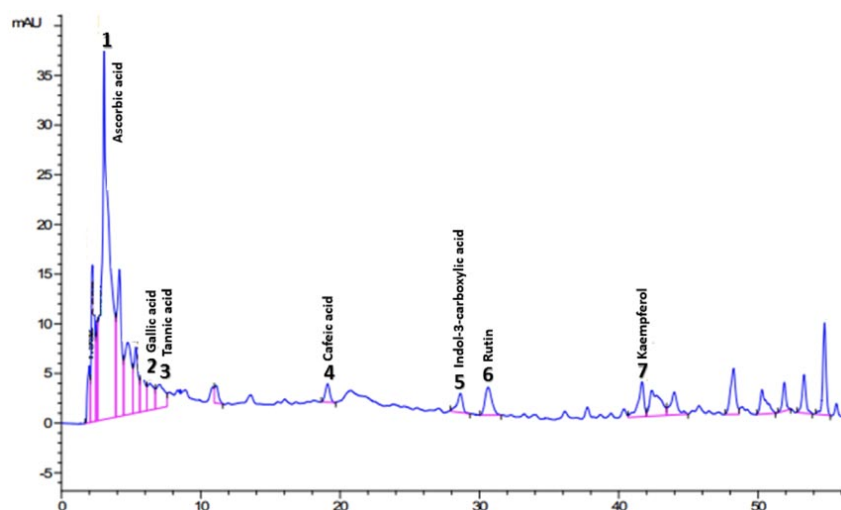


Fig. 1. Phenolic compounds of aqueous extract from ginger at 254 nm. Pic 1: Ascorbic acid, Pic 2: Galic acid, Pic 3: Tannic acid, Pic 4: Cafeic acid, Pic 5: Indol-3-carboxylic acid, Pic 6: Rutin, Pic 7: Kaempferol.

A total of seven phenolic compounds were detected, belonging to two classes: phenolic acids and flavonoids. Among these, two flavonol glycosides (peaks 6 & 7) were identified.

The main identified compounds included:

- Peak 1: Ascorbic acid, confirmed by its retention time (3.055 min) and ultra-violet (UV) spectrum.
- Peak 2: Gallic acid.
- Peak 3: Tannic acid, identified through comparison with the standard.
- Peak 4: Caffeic acid, with a retention time of 19.102 min.
- Peak 5: Indole-3-carboxylic acid, at 28.633 min.
- Peak 6: Rutin.
- Peak 7: Kaempferol.

These findings highlight the presence of diverse phenolic compounds and flavonoids in the aqueous ginger extract, with variations in concentration across the identified molecules.

Body Weight. Figure 2A illustrates the changes in body weight over the course of the experiment. The results indicate that both groups I (control) and II (HFD-fed) experienced a gradual increase in body weight over seven weeks, with significant peaks at weeks 3, 4, and 6. Notably, group II exhibited a markedly higher weight gain compared to group I, with a significant increase of approximately 103.07 % versus 54.30 %, respectively, by the end of the study. Treatment with *Zingiber officinale* for three weeks (group III) significantly reduced the percentage of weight gain compared to group II (Fig. 2A). By the end of the experiment, group III's body weight gain was lower than that of the control group, with a reduction of about 56.46 %, bringing it to a level comparable to group I.

Lipid Profile. Rabbits in group II (HFD) showed an insignificant increase in plasma triglyceride (TG) levels compared to controls, with a rise of approximately 45.35 % (Fig. 2B). Conversely, TG levels significantly decreased in group III, with a reduction of 44.12 % relative to group II ($p < 0.05$). One-way ANOVA confirmed significant differences among the groups.

Total cholesterol (TC) levels were significantly reduced in group II by about 34.33 % compared to controls (Fig. 2C). In group III, TC levels increased slightly (~13.64 %) compared to group II; however, this change was not statistically significant. ANOVA analysis indicated significant differences ($p < 0.05$).

HDL cholesterol (HDLc) levels did not differ significantly between groups I and II. In group III, HDLc increased by approximately 25 % relative to group II, though this was not statistically significant (Fig. 2D).

LDL cholesterol (LDLc) levels were significantly lower in group II (by 19.35 %) compared to controls (Fig. 2E). Treatment with ginger powder (group III) resulted in a non-significant increase in LDLc levels (16.00 %) compared to group II. Overall, the differences among the three groups were statistically significant ($p < 0.05$) according to ANOVA.

Hormonal Profile. In rabbits fed a high-fat diet (HFD, Group II), there was an observed increase in free T4 (FT4) levels compared to the control group (Group I), as shown in Figure 2F. However, this increase was not statistically significant and was approximately 27.5 %. Group III exhibited a slight increase in FT4 levels relative to Group II, which was also not significant and was around 21.5 %. Overall, the differences among the three groups were statistically significant ($p < 0.05$), as determined by one-way ANOVA.

Regarding cortisol levels, Group II showed a decrease of about 55 %, though this change was not statistically significant compared to Group I (Fig. 2G). Conversely, Group III displayed an increase in plasma cortisol levels of approximately 107 %, but again, this difference was not significant when compared to Group II.

Correlation Analysis Between Hormone Levels and Lipid Profiles. Analysis of the correlations presented in Table III reveals several significant relationships between thyroid hormones, cortisol, and lipid parameters across the different experimental groups.

In rabbits fed a high-fat diet (HFD), FT4 levels exhibited a notable negative correlation with triglycerides ($r = -0.34$, $p < 0.05$), indicating that higher FT4 corresponds to lower triglyceride levels in this group. Conversely, in the group treated with *Zingiber officinale*, this relationship was not significant ($r = +0.19$). Additionally, within the same group, no significant positive correlation was observed between FT4 and cholesterol levels ($r = +0.1$), whereas in the group receiving the herbal treatment, a significant positive correlation emerged ($r = +0.3$), suggesting a potential modulatory effect.

Regarding lipid fractions, a significant negative correlation was identified between FT4 and HDL cholesterol in group III ($r = -0.37$, $p < 0.05$), implying that increased FT4 is associated with decreased HDL levels in this context. Similarly, FT4 showed a highly significant negative correlation with HDL cholesterol in this group ($r = -0.26$). For LDL cholesterol, a positive correlation was observed in HFD-fed rabbits ($r = +0.19$, $p < 0.05$), indicating that higher FT4 levels may correspond to elevated LDL. Interestingly, in group III, this association was not significant ($r = -0.044$), suggesting that the relationship may be context-dependent.

Regarding cortisol, no significant correlations were found with triglycerides in HFD-fed rabbits ($r = -0.025$) or in group III ($r = +0.199$). Similarly, cortisol levels did not significantly correlate with total cholesterol in group II ($r = -0.58$) or in rabbits treated with *Zingiber officinale* ($r = +0.1784$). At the end of the study, cortisol levels showed no significant association with HDL cholesterol in groups II ($r = -0.49$) and III ($r = -0.26$), nor with LDL cholesterol (group II: $r = -0.50$; group III: $r = -0.29$). These findings suggest that, within the experimental conditions, cortisol does not markedly influence lipid parameters.

Overall, these results highlight complex, hormone-specific interactions with lipid profiles, with notable differences depending on diet and treatment, emphasizing the modulatory role of thyroid function and stress hormones in lipid metabolism under high-fat dietary conditions.

Table III. Relationship between Lipid and Hormones in Rabbits Fed HFD and Rabbits Treated with *Zingiber officinale*

Correlations	Group II	P- value	Group III	P-value
FT4 levels and TG levels	$r = - 0.34^{**}$	$P=0.0013$	$r = +0.19$	$P=0.8633$
FT4 levels and TC levels	$r = +0.1$	$P=0.0656$	$r = +0.30^*$	$P=0.0205$
FT4 and HDL-C	$r = -0.37^{**}$	$P=0.0094$	$r = - 0.26^{***}$	$P=0.0009$
FT4 and LDL-C	$r = +0.19^*$	$P=0.0085$	$r = - 0.044^{**}$	$P=0.0043$

The results are the coefficient of correlation values r. FT4: free thyroxine, triglyceride: TG; total cholesterol: TC; High density lipoprotein cholesterol: HDLc; low density lipoprotein cholesterol: LDLc; (n= 9); r: Pearson correlation. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

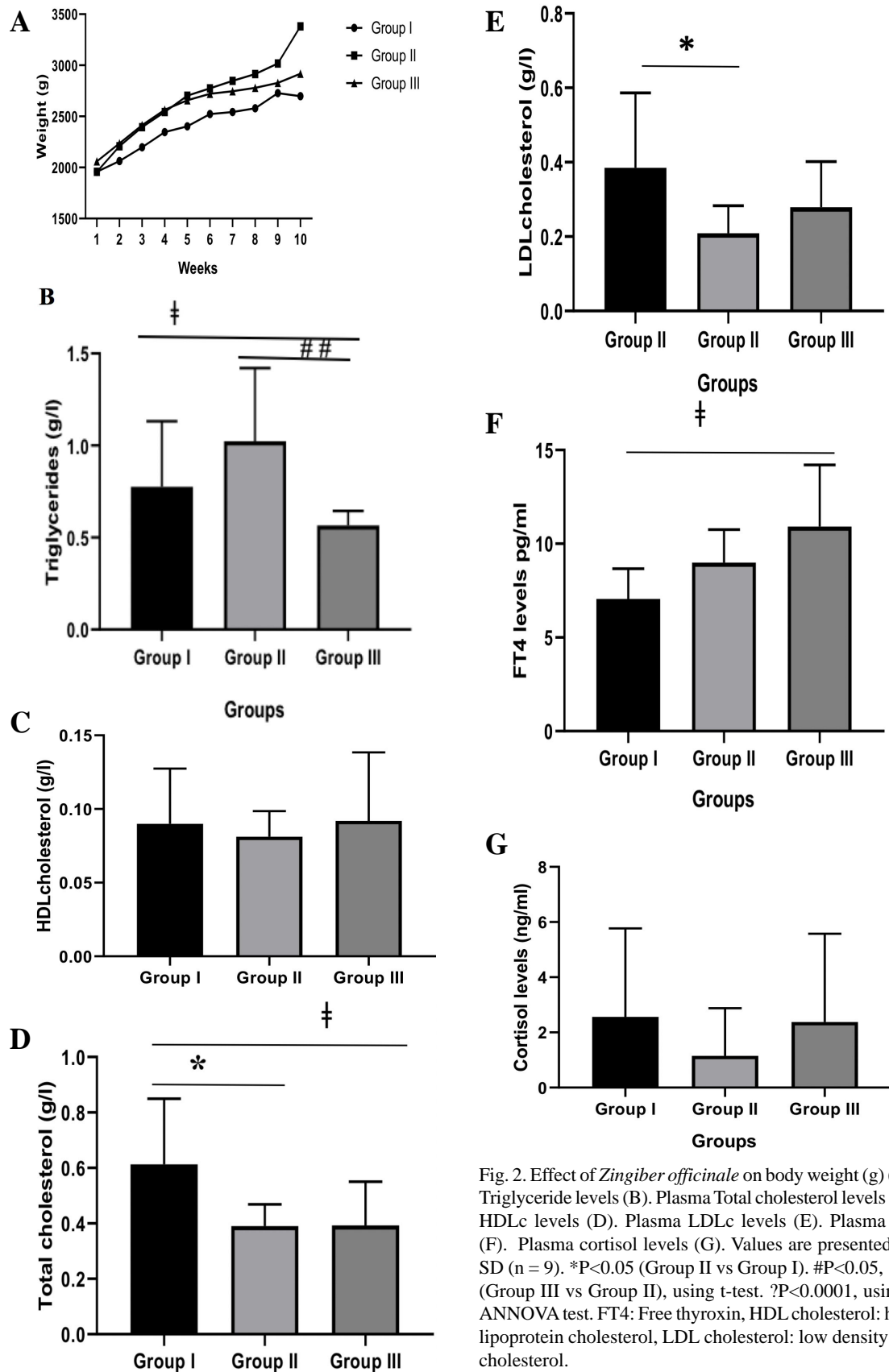


Fig. 2. Effect of *Zingiber officinale* on body weight (g) (A). Plasma Triglyceride levels (B). Plasma Total cholesterol levels (C). Plasma HDLc levels (D). Plasma LDLc levels (E). Plasma FT4 levels (F). Plasma cortisol levels (G). Values are presented as mean \pm SD (n = 9). *P<0.05 (Group II vs Group I). †P<0.05, ††P<0.001 (Group III vs Group II), using t-test. †††P<0.0001, using one-way ANNOVA test. FT4: Free thyroxine, HDL cholesterol: high density lipoprotein cholesterol, LDL cholesterol: low density lipoprotein cholesterol.

Morphometric measurements. The colloid area increased in rabbits fed a high-fat diet (HFD), with an approximate 97 % increase compared to the control group (Group I); however, this change was not statistically significant. Treatment with *Zingiber officinale* (Group III) resulted in an insignificant reduction in colloid area (~30.31 % decrease compared to Group II).

The follicular size showed a non-significant increase in Group II compared to controls (about 11.08 %). In contrast, Group III exhibited a significant decrease in follicular size relative to Group II, with a reduction of approximately 11.24%.

The average size of the largest follicles was significantly higher in Group II than in controls, representing an increase of approximately 78.13 %. Ginger treatment (Group III) significantly reduced the size of these follicles compared to Group II, with a decrease of about 33.26 %.

Similarly, the smallest follicles in the HFD group (Group II) increased significantly compared to controls, showing a 35.53 % rise. This increase was significantly mitigated in Group III, which experienced a 14.22 % reduction relative to Group II (Table IV).

Histological Analysis of the Thyroid Gland. In control rabbits, Haidenhain Azan-stained sections revealed numerous, regularly shaped follicles of varying sizes (Fig. 3A). These follicles were lined by cuboidal follicular epithelium with rounded nuclei, and their lumens were filled with homogeneous, moderate colloid characteristic of an euthyroid state, with peripheral vacuoles observed within the colloid. The follicles were separated by connective tissue containing collagen fibers, blood capillaries with flattened endothelial cells, and red blood cells. Normal parafollicular cells were identifiable between the cuboidal follicular cells and interspersed among follicles.

In contrast, the thyroid glands of HFD-fed rabbits displayed significant histopathological alterations, indicative of disrupted architecture. Follicular cells appeared markedly disorganized and flattened (Fig. 3B), while others exhibited a

rounded morphology. Some follicular cells showed increased staining intensity, indicating possible cellular activity or pathology. Additionally, constricted follicular structures were observed, suggesting alterations in normal follicular architecture. These findings highlight notable histological changes in the thyroid tissue.

Cytoplasmic lipid droplets and basement membrane rupture were also observed in some follicular cells. Inter-follicular cell hypertrophy was evident, reflecting epithelial proliferation, while nuclei exhibited darkly stained (pyknosis) characteristics (Fig. 3C). Several follicles were dilated, containing non-homogeneous colloids, with some showing decreased colloid content and peripheral vacuolation; certain follicles appeared empty and lacked intact follicular walls (Fig. 3C). Hemorrhage and extensive fibrosis within the thyroid parenchyma were notable (Fig. 3C), alongside the presence of pyknotic nuclei in follicular cells (Fig. 3D).

Additionally, the tissue demonstrated dilated and congested blood capillaries (Fig. 3E,F), with infiltration of inflammatory cells—including mast cells, plasma cells, monocytes, and lymphocytes—distributed within the connective tissue (Fig. 3D). Lesions affecting follicular epithelium and basement membrane integrity were evident, with lipid droplets prominently observed in HFD-fed rabbits (Fig. 3F).

Sections from rabbits treated with ginger rhizome powder showed remarkable preservation of thyroid architecture. The gland nearly retained its normal lobular pattern, with follicles filled with colloid and containing vacuoles (Fig. 3G), and follicular cells exhibiting typical morphology. The interfollicular connective tissue was less prominent, with reduced fibrosis and minimal inflammatory infiltration (Fig. 3H). Blood capillaries appeared normal and uncongested, with the absence of hemorrhage (Fig. 3G,H). Some follicles contained reduced colloid, with flattened thyrocytes and areas of follicular epithelium loss (Fig. 3H), and the interfollicular spaces were narrowed. A few follicles exhibited minimal colloid content and peripheral vacuolation (Fig. 3G).

Table IV. Morphometric Measurements (µm) of Thyroid Gland in Different Groups.

Measurements	Group I	Group II	Group III
Area of colloid (µm ²)	2368.52±277.04	4677.45±535.98	2859.67±240.66
Follicular Size (µm)	63.29±3.51	70.35±2.32	6244±2.14 ⁺
Size of largest follicles (µm)	94.48±3.55	168.30±5.61 ^{***}	112.32± 3.43 ⁺⁺⁺
Size of smallest follicles (µm)	43.39±2.52	60.11±1.61 ^{***}	51.56±1.38 ⁺⁺⁺

The results are the mean ± standard deviations. Statistical analysis was performed using t-test. * : vs control; +: vs HFD. *, +: p<0.05; **,++: p<0.01; ***,+++ : p<0.001; ****,++++: p<0.0001. Group I : Control rabbits ; Group II : High Fat Diet rabbits; Group III : *Zingiber officinale* treated rabbits. A total of 100 measurements were performed for area of colloid, follicular size, size of largest follicles and size of smallest follicles in control, HFD and *Zingiber officinale* rabbits.

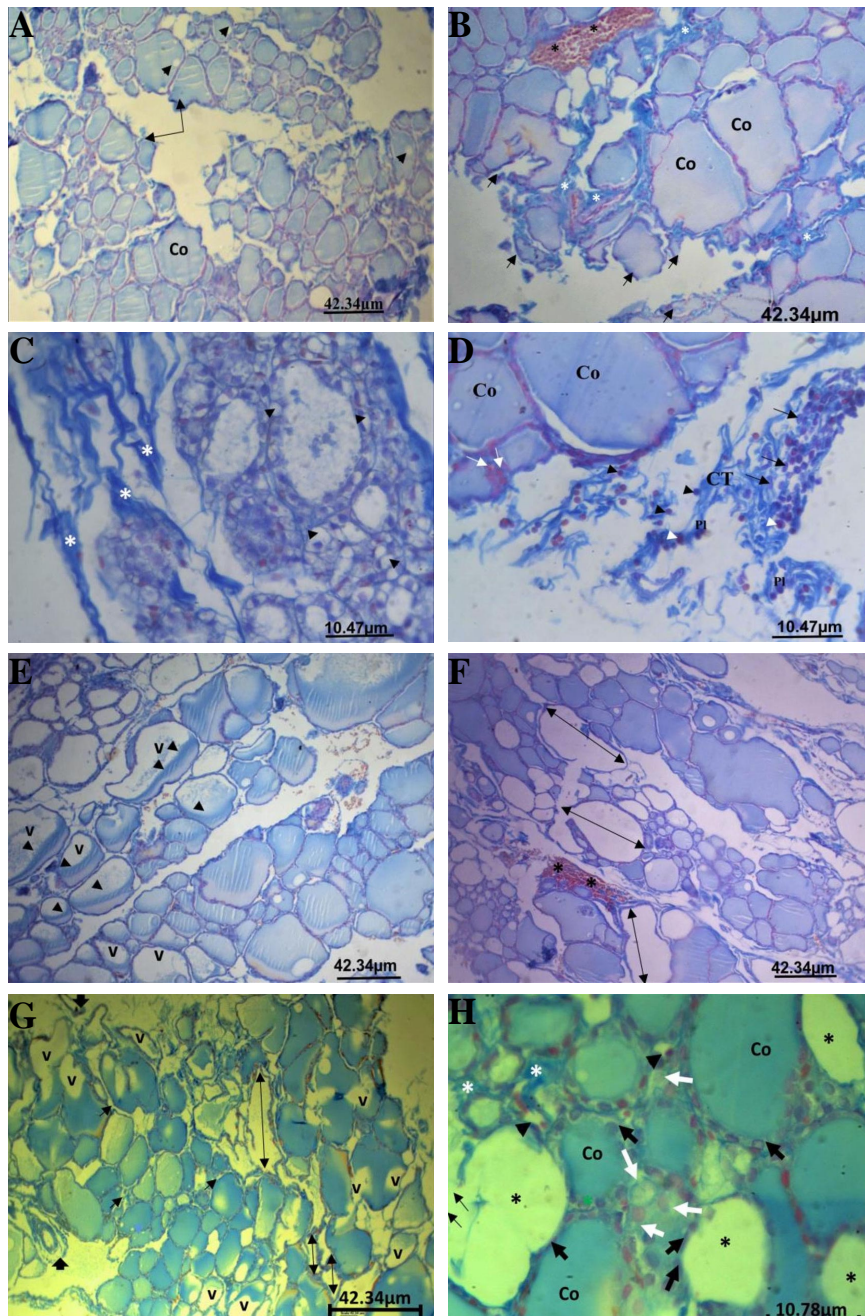


Fig. 3. Histology of the thyroid gland in control rabbits: Group I (A), HFD rabbits: Group II (B, C, D, E, F) and *Zingiber officinale* treated rabbits: Group III (G, H) Rabbit: *Oryctolagus cuniculus*. The cross sections are stained with Heidenhain's Azan. Co: colloid. CT: Connective Tissue. Pl: plasma cells. Vs: vacuolated colloid. The thyroid gland of control rabbits (A) showed a normal structure, variable, regular and rounded follicles (long black arrows), the colloid showed homogenous structure; containing vacuoles (black head arrows). In HFD rabbits (B, C, D, E, F), we showed the degenerated follicles with a loss of their epithelium lining (black arrows), disorganization of interstitial tissue and we observed an accumulation of collagen fibers between follicles that corresponds to fibrosis (white stars), and notice within the connective tissue appears hemorrhage (black stars), some thyreocytes exhibited intracellular lipid droplets (black head arrows), the follicular cells contained small darkly stained nuclei or pycnotic cells (white arrows), disorganization of connective tissue (black arrows), Important leukocyte infiltration is reported in the interstitial tissue such as lymphocyte (black head arrows), monocytes (white head arrows) and mast cells (black head arrows), follicles showed retracted colloid (black head arrows), few follicles empty of colloid and devoid of epithelial wall (arrows in both directions). The thyroid gland of treated rabbits with *Zingiber officinale* (G, H) demonstrated an endocrine tissue that appears formed with normal and similar to the usual lobular structure showing narrow inter-follicular spaces (black arrows), the connective tissue contain normal blood capillaries black head arrows, few follicles empty of colloid and devoid of epithelial wall (arrows of two senses), others showing flattened follicular cells (black arrows), follicles devoid of follicular epithelium long (black star); with normal blood capillaries (black head arrows), less intense fibrosis (white stars).

These observations indicate that HFD induces notable thyroid tissue damage characterized by disorganization, lipid accumulation, inflammatory infiltration, hemorrhage, and fibrosis, which can be ameliorated by ginger treatment.

Liver Histology. Representative images of liver samples from each rabbit group are shown in Figure 4, highlighting the central vein surrounded by hepatocyte layers. In rabbits fed a standard diet, the liver tissue displayed normal architecture with appropriate collagen distribution (Fig. 4A). Conversely, rabbits subjected to a high-fat diet (HFD)

exhibited key features of non-alcoholic steatohepatitis (NASH), including microvesicular steatosis, hepatocellular ballooning, small deeply stained (pycnotic), focal necrosis (Fig. 4D), hyperemia in sinusoids and central veins (Fig. 4B,D), and inflammatory infiltration (Fig. 4B).

Using Haidenhain stain, extensive collagen fiber deposition was observed in HFD-fed rabbits, indicating fibrosis that distorted the collecting bile ducts structure (Fig.4C) and signifying progressive hepatic damage (Fig. 4D). In contrast, liver tissue from rabbits treated with ginger

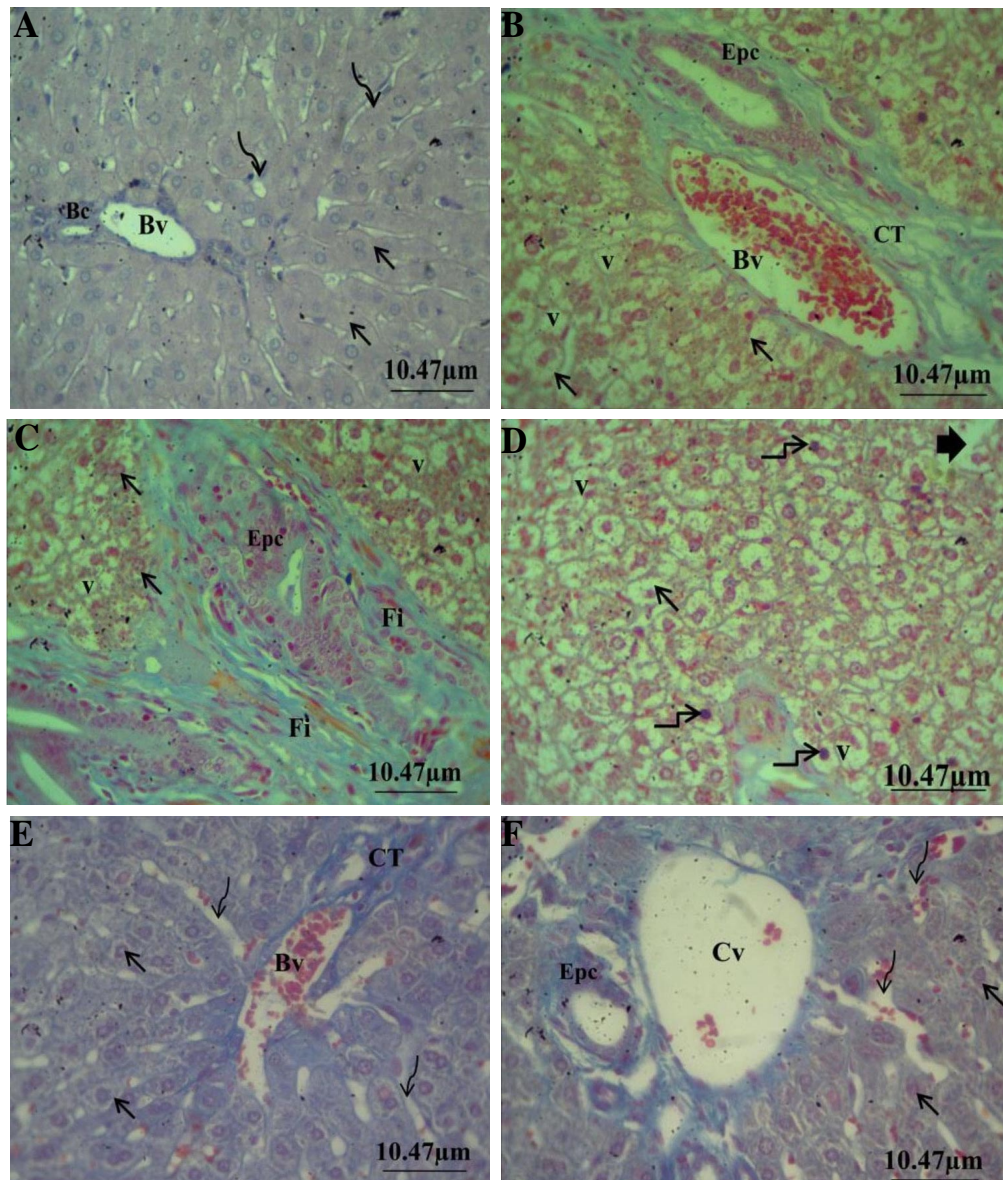


Fig. 4. Histology of the liver in control rabbits: Group I (A). HFD rabbits: Group II (B, C, D) and *Zingiber officinale* treated rabbits: Group III (E, F). Rabbit: *Oryctolagus cuniculus*. The transverse sections are stained with Heidenhain's Azan. CV: Central Vein. CB-the Collecting Bile ducts. Epc : Epithelial cells. CT: Connective Tissue. Vs: microvesicular steatosis. Bv: congested Blood vessel. Fi: Fibrosis. The hepatic tissue of control rabbits (A) contains liver cells or hepatocytes (small black arrows), are epithelial cells organized into interconnected cords arranged radially around the central vein. The spaces between these cords form sinusoids, which are capillary-like structures composed of a discontinuous layer of fenestrated endothelial cells. The collecting bile ducts, which vary in size, are lined with simple cuboidal or columnar epithelium. The High-Fat diet (B, C, D) showing histologic assessment of liver damage revealed after high fat diet consumption; the collecting bile ducts contain epithelial cells. Notice the excessive connective tissue or fibrosis around the collecting bile ducts. The hepatocyte cells (black arrows) show microvesicular steatosis and blood vessel appears congested. The collecting bile ducts is lined by cuboidal epithelial cells, their lumen appears narrow with disorganized epithelial cells. Notice fibrosis surround and constrict the collecting bile ducts, the hepatocytes contain microvesicular steatosis. The hepatocytes have pyknotic cells (small black arrows), others show apoptotic appearance (black arrows), some hepatocyte cells have fatty change in leading to the appearance of steatosis (head arrows), showing focal necrosis sites. The *Zingiber officinale* rabbits (E, F) assessment of normal histological liver structure after treatment with ginger: The liver tissue shows restoration of a hepatic architecture, most of liver cells or hepatocytes have normal shape and vesicular nuclei, with basophilic cytoplasm. Noticed disappearance of microvesicular steatosis with few hepatic veins congestion. The hepatic tissue shows a regular collecting bile ducts structure, which is lined with layer cubic epithelial cells and wide lumen, with disappearance of fibrosis, the central vein is surrounded by fine connective tissue.

powder showed significant improvement, with near-normal architecture. The hepatic tissue appeared largely regenerated, characterized by reorganization of hepatocyte morphology, cytoplasmic and plasma membrane restoration, reduced lipid accumulation, and diminished granular changes within hepatocytes. Additionally, lobular architecture and the collecting bile ducts structure were improved, with persistent blood congestion but absence of fibrosis (Fig. 4E,F).

Immunohistochemical Study of the Thyroid Gland.

Immunohistochemical analysis of thyroid sections revealed that TTF-1, a transcription factor involved in regulating the expression of Tg, TPO, and NIS, was predominantly localized in the nucleus. The intensity of TTF-1 staining was markedly reduced in the HFD group (Fig. 5B) compared to the control and *Zingiber officinale*-treated groups, where staining was notably stronger (Fig. 5A,C).

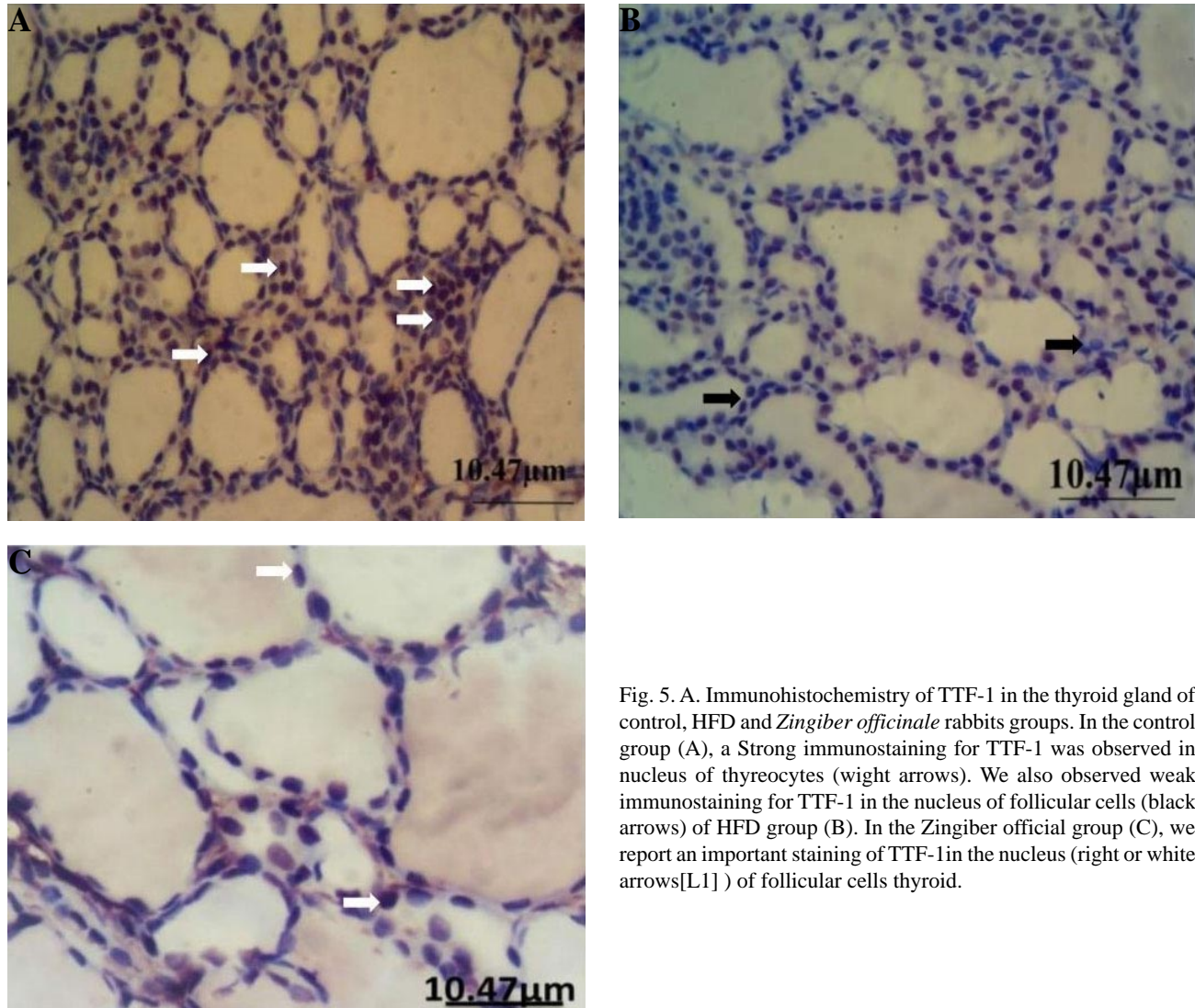


Fig. 5. A. Immunohistochemistry of TTF-1 in the thyroid gland of control, HFD and *Zingiber officinale* rabbits groups. In the control group (A), a Strong immunostaining for TTF-1 was observed in nucleus of thyrocytes (white arrows). We also observed weak immunostaining for TTF-1 in the nucleus of follicular cells (black arrows) of HFD group (B). In the *Zingiber officinale* group (C), we report an important staining of TTF-1 in the nucleus (white arrows) of follicular cells thyroid.

DISCUSSION

This study investigated the effects of a hyperlipidic diet (HFD) on metabolic parameters, thyroid and liver histology, and the potential protective role of ginger phenolics in rabbits. The findings reveal significant diet-induced alterations and demonstrate ginger's capacity to mitigate these effects through its rich phenolic content.

The aqueous ginger extract exhibited substantial levels of phenolic compounds, with total phenolics (TPC) of 8.3 ± 0.36 mg GAE/g and total flavonoids (TFC) of 4.17 ± 0.2 mg quercetin equivalents/g. These values are consistent with previous reports Seo *et al.* (2021), confirming ginger's status as a rich source of bioactive phenolics. High-

performance liquid chromatography (HPLC) analysis identified major phenolic acids, particularly ascorbic acid (vitamin C, 34.25 %), gallic acid, tannic acid, caffeic acid, and indole-3-carboxylic acid. Among flavonoids, kaempferol (2.81 %) and rutin (2.55 %) were predominant, aligning with literature reports (Iftikhar *et al.*, 2022). These compounds are well-known for their antioxidant, anti-inflammatory, and lipid-modulating properties.

Subjected to HFD exhibited significant weight gain over ten weeks, likely due to increased caloric intake from saturated fats promoting adiposity. This observation aligns with Seo *et al.* (2021), who reported ginger's anti-obesity effects via suppression of adipogenesis and enhancement of fatty acid oxidation. The presence of antioxidants such as vitamin C and rutin could have contributed to attenuating excessive weight gain.

Lipid profile assessments showed a significant increase in serum triglycerides (TGs), consistent with previous findings (Saravanan *et al.*, 2014). Elevated TGs may result from augmented gastric lipase activity and increased intestinal fat absorption. Interestingly, total cholesterol, LDL, and HDL cholesterol levels decreased significantly in HFD-fed rabbits, a pattern also observed by Elrokh *et al.* (2010). This unexpected decrease might be attributable to complex lipid regulatory responses or diet-specific factors, but it suggests that ginger's phenolic compounds—such as rutin and kaempferol—may influence lipid metabolism by modulating lipase activity and lipid clearance (Anoh *et al.*, 2022).

The impact of HFD on thyroid hormones was subtle; plasma FT4 levels increased slightly but insignificantly. Similar findings were reported by Araujo *et al.* (2010), indicating that lipid-rich diets may induce minor alterations in thyroid hormone levels without significant changes in T3 or T4. Notably, ginger supplementation significantly increased FT4 levels, consistent with reports by Habeeb *et al.* (2019), who observed elevated thyroid hormones following ginger administration.

Serum cortisol levels decreased by approximately 55 % in HFD-fed rabbits, although this change lacked statistical significance. Ginger treatment caused an insignificant yet notable increase (~107 %) in cortisol, suggesting a possible modulatory effect on stress hormones deserving further exploration.

Correlation analyses showed that FT4 was negatively associated with serum triglycerides and LDL cholesterol, and positively associated with total cholesterol. These relationships mirror findings by Jang *et al.* (2018) and Roos

et al. (2007), emphasizing the interplay between thyroid function and lipid metabolism. Reduced FT4 levels are often linked with dyslipidemia and metabolic disturbances, whereas restoring thyroid hormone levels may improve lipid profiles.

Histologically, the thyroid glands of HFD-fed rabbits displayed irregular, dilated follicles lined with flattened epithelium and abundant colloid, indicative of glandular inactivity or hypothyroidism. Additionally, cytoplasmic vacuolation and small deeply stained nuclei (Pyknosis) of follicular cells signified cellular degeneration, likely driven by lipotoxicity, oxidative stress, and inflammation. Similar alterations have been documented in rats fed HFD (El-Sayed & Ibrahim, 2020).

Inflammatory infiltration, including mast cells, plasma cells, lymphocytes, and fibrosis, was evident, suggesting tissue inflammation consistent with obesity-related thyroid dysfunction [30]. Vascular congestion and follicular fusion indicated structural disruption, possibly resulting from oxidative damage and impaired microcirculation.

Remarkably, ginger supplementation ameliorated these histological damages, restoring normal follicular architecture, reducing vacuolation, and normalizing tissue structure. The antioxidant properties of ginger's phenolics—such as 6-gingerol, 6-shogaol, caffeic acid, rutin, and quercetin—likely underlie these protective effects by scavenging free radicals, reducing lipid peroxidation, and suppressing inflammation (Silva *et al.*, 2010; Omshi *et al.*, 2018). Immunohistochemically, TTF-1 expression, which was downregulated in HFD groups, was significantly upregulated following ginger treatment. Since TTF-1 is crucial for thyroid-specific gene transcription, its restoration suggests improved glandular function.

The liver of HFD-fed rabbits exhibited fatty infiltration and cellular degeneration, consistent with steatosis. Ginger's phenolic compounds, particularly vitamin C, exert hepatoprotective effects by attenuating oxidative stress, reducing lipid accumulation, and suppressing inflammatory responses (Tzeng *et al.*, 2015). These effects contribute to the normalization of hepatic histology observed in ginger-treated groups.

CONCLUSIONS

Overall, a hyperlipidic diet induces significant metabolic disturbances, including dyslipidemia, hormonal imbalance, and tissue degeneration in the thyroid and liver. Ginger's phenolic constituents demonstrated promising protective effects by improving tissue structure, modulating

lipid and hormone profiles, and enhancing TTF-1 expression. These findings support the potential of ginger as a natural adjunct therapy for diet-induced metabolic and tissue dysfunctions.

Future research should focus on isolating specific bioactive compounds, elucidating their molecular mechanisms, and exploring the signaling pathways involved in their anti-inflammatory and antioxidant actions. Additionally, longer-term studies with varied doses could better define optimal therapeutic strategies.

BIREM, Z.; AMARA, N.; TOUATI, S.; OMARI, N. & KOCEIR, E-H.A. Efectos beneficiosos del polvo de rizoma de *Zingiber officinalis* sobre la tiroides y el hígado de conejos machos alimentados con una dieta rica en grasas. *Int. J. Morphol.*, 44(2):480-492, 2026.

RESUMEN: El aceite de palma, rico en ácidos grasos, se utiliza ampliamente en la industria alimentaria. El consumo excesivo de dietas ricas en grasas (DRG) puede provocar la acumulación ectópica de lípidos, afectando negativamente la estructura y función de los tejidos, especialmente en el hígado y la tiroides. El jengibre (*Zingiber officinale*) posee diversas propiedades biológicas, incluyendo efectos antioxidantes, antiinflamatorios e hipolipemiantes. Este estudio tuvo como objetivo investigar si el polvo de jengibre ejerce efectos protectores contra las alteraciones lipídicas y hormonales inducidas por una DRG y su impacto en la arquitectura celular de la tiroides y el hígado en conejos machos. Se cuantificaron los contenidos totales de fenoles y flavonoides, y se identificaron los compuestos fenólicos mediante cromatografía líquida de alta resolución (HPLC). Se midieron los niveles hormonales utilizando ensayos apropiados. Para evaluar los cambios celulares se realizaron exámenes histológicos de los tejidos tiroideo y hepático, con inmunohistoquímica aplicada específicamente al tejido tiroideo. La suplementación con jengibre demostró efectos protectores al mejorar los perfiles lipídicos y hormonales y restaurar la arquitectura celular en ambos tejidos. Histológicamente, las células mostraron una reducción de la vacuolización citoplasmática y se observó el restablecimiento de los capilares sanguíneos, junto con la desaparición de las hemorragias. El alto contenido de vitamina C en el jengibre probablemente contribuyó a su actividad antioxidante, protegiendo los tejidos del estrés oxidativo. Los compuestos fenólicos, incluyendo rutina, kaempferol y ácido cafeico, parecieron mediar estos efectos al disminuir la fibrosis y la inflamación. Estos hallazgos sugieren que el jengibre tiene efectos protectores significativos contra las alteraciones tisulares inducidas por una dieta rica en grasas, principalmente a través de sus propiedades antioxidantes y sus componentes fenólicos. Los resultados aportan información sobre los mecanismos mediante los cuales los compuestos fenólicos mitigan la fibrosis y la inflamación. Futuras investigaciones deberían explorar el potencial terapéutico de compuestos específicos como la rutina, el kaempferol, la vitamina C y el ácido cafeico para proteger los tejidos tiroideo y hepático en condiciones de estrés metabólico.

PALABRAS CLAVE: *Zingiber officinale* / Compuestos fenólicos; Función tiroidea; Función hepática; Metabolismo lipídico; Tiroxina-cortisol libre.

REFERENCES

- Anoh, K. U.; Archibong, E. E.; Nsa, E. E.; Udoekong, E. C. & Jimmy, N. P. Antioxidant activities of vitamin C and bicarbonate buffers on hormone secretion and serum metabolites of heat-stressed rabbit buck. *Ann. Res. Rev. Biol.*, 37(1):59-68, 2022.
- Apovian, C. M.; Aronne, L. J.; Bessesen, D. H.; McDonnell, M. E.; Murad, M. H.; Pagotto, U.; Ryan, D. H. & Still, C. D. Pharmacological management of obesity: an Endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab.*, 100(2):342-62, 2015.
- Araujo, R. L.; Andrade, B. M.; Padrón, A. S.; Gaidhu, M. P.; Perry, R. L. S.; Carvalho, D. P. & Ceddia, R. B. High-fat diet increases thyrotropin and oxygen consumption without altering circulating 3,5,3 ϵ -triiodothyronine (T3) and thyroxine in rats: the role of iodothyronine deiodinases, reverse T3 production, and whole-body fat oxidation. *Endocrinology*, 151(7):3460-9, 2010.
- Auvinen, H. E.; Romijn, J. A.; Biermasz, N. R.; Pijl, H.; Havekes, L. M.; Smit, J. W.; Rensen, P. C. & Pereira, A. M. The effects of high-fat diet on the basal activity of the hypothalamus-pituitary-adrenal axis in mice. *J. Endocrinol.*, 214(2):191-7, 2012.
- Boursier, C. M.; Bosco, D.; Coulibaly, A. & Negre, M. Are traditional neem extract preparations as efficient as a commercial formulation of azadirachtin A? *Crop Prot.*, 30(3):318-22, 2011.
- Bravo, E.; Palleschi, S.; Aspichueta, P.; Buqué, X.; Rossi, B.; Cano, A.; Napolitano, M.; Ochoa, B. & Botham, K. M. High-fat diet-induced non-alcoholic fatty liver disease in rats is associated with hyperhomocysteinemia caused by down-regulation of the trans-sulphuration pathway. *Lipids Health Dis.*, 10:60, 2011.
- Camasta, S.; Manco, M.; Frascerra, S.; Iaconelli, A.; Mingrone, G. & Ferrannini, E. Day-long pituitary hormones in morbid obesity: effects of bariatric surgery. *Int. J. Obes. (Lond)*, 33(1):166-72, 2009.
- Chakraborty, T. R.; Donthireddy, L.; Adhikary, D. & Chakraborty, S. Long-term high-fat diet has a profound effect on body weight, hormone levels, and estrous cycle in mice. *Med. Sci. Monit.*, 22:1601-8, 2016.
- Crozier, A.; Lean, M. E. J.; McDonald, M. S. & Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *J. Agric. Food Chem.*, 45(3):590-5, 1997.
- Elrokh, E. S.; Yassin, N. A. Z.; El-Shenawy, S. M. A. & Ibrahim, B. M. M. Antihypercholesterolaemic effect of ginger rhizome (*Zingiber officinale*) in rats. *Inflammopharmacology*, 18(6):309-15, 2010.
- El-Sayed, S. M. & Ibrahim, H. M. Effect of high-fat diet-induced obesity on thyroid gland structure in female rats and the possible ameliorating effect of metformin therapy. *Folia Morphol. (Warsz.)*, 79(3):476-88, 2020.
- Gabe, M. *Techniques Histologiques*. Paris, Masson, 1968.
- Habeeb, A. A.; El-Darawany, A. A.; Nasr, A. S. & Sharaf, A. K. Impact of some medicinal plant supplements on pregnant rabbit diet during hot summer season. *Res. J. Med. Plants*, 13(4):145-54, 2019.
- Huang, C. N.; Horng, J. S. & Yin, M. C. Antioxidative and antiglycative effects of six organosulfur compounds in low-density lipoprotein and plasma. *J. Agric. Food Chem.*, 52(11):3674-8, 2004.
- Ifitkhar, N.; Hussain, A. I.; Chatha, S. A. S.; Sultana, N. & Rathore, H. A. Effects of polyphenol-rich traditional herbal teas on obesity and oxidative stress in rats fed a high-fat-sugar diet. *Food Sci. Nutr.*, 10(3):698-711, 2022.
- Jang, J.; Kim, Y.; Shin, J.; Lee, S. A.; Choi, Y. & Park, E. C. Association between thyroid hormones and the components of metabolic syndrome. *BMC Endocr. Disord.*, 18(1):29, 2018.
- Kaplan, L. A. & Pesce, A. J. *Triglycerides*. In: Kaplan, L. A. & Pesce, A. J. (Eds.). *Clinical Chemistry*. 2nd ed. St. Louis (MO), Mosby, 1984. pp.1207-13.
- Kyrou, I. & Tsigos, C. Stress hormones: physiological stress and regulation of metabolism. *Curr. Opin. Pharmacol.*, 9(6):787-93, 2009.
- Naito, H. K. & Kaplan, A. Q. *High-density lipoprotein (HDL) cholesterol*. In: Kaplan, L. A. & Pesce, A. J. (Eds.). *Clinical Chemistry*. 2nd ed. St. Louis (MO), Mosby, 1984. pp.1207-13.

- Omshi, F. S.; Abbasalipourkabir, R.; Abbasalipourkabir, M.; Nabyan, S.; Bashiri, A. & Ghafourikhosroshahi, A. Effect of vitamin A and vitamin C on attenuation of ivermectin-induced toxicity in male Wistar rats. *Environ. Sci. Pollut. Res. Int.*, 25(29):29408-17, 2018.
- Roos, A.; Bakker, S. J. L.; Links, T. P.; Gans, R. O. B. & Wolffenbuttel, B. H. R. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *J. Clin. Endocrinol. Metab.*, 92(2):491-6, 2007.
- Saravanan, G.; Ponmurugan, P.; Deepa, M. A. & Senthilkumar, B. Anti-obesity action of gingerol: effect on lipid profile, insulin, leptin, amylase and lipase in male obese rats induced by a high-fat diet. *J. Sci. Food Agric.*, 94(14):2972-7, 2014.
- Silva, T.; Oliveira, C. & Borges, F. Caffeic acid derivatives, analogs and applications: a patent review (2009–2013). *Expert Opin. Ther. Pat.*, 24(11):1257-70, 2014.
- Sinha, R.; Singh, B. K. & Yen, P. M. Direct effects of thyroid hormones on hepatic lipid metabolism. *Nat. Rev. Endocrinol.*, 14(5):259-69, 2018.
- Seo, S. H.; Fang, F. & Kang, I. Ginger (*Zingiber officinale*) attenuates obesity and adipose tissue remodeling in high-fat diet-fed C57BL/6 mice. *Int. J. Environ. Res. Public Health*, 18(2):631, 2021.
- Tzeng, T.; Liou, S.; Chang, C. J. & Liu, I. Gingerol dampens hepatic steatosis and inflammation in experimental nonalcoholic steatohepatitis. *Phytomedicine*, 22(4):452-61, 2015.
- Williamson, G. & Carughi, A. Polyphenol content and health benefits of raisins. *Nutr. Res.*, 30(8):511-9, 2011.

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