

Original Research Article

Exploring the therapeutic potential of phenolic compounds and endurance training in ameliorating heart dysfunction induced by hypothyroidism: insights into *Sirt-1/Nrf2/IL-6/Myh7/Col1a2* axis and lncRNAs

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Abstract

Objective: Dysregulation of thyroid hormones is a significant contributor to cardiac dysfunction, yet the precise mechanisms linking hypothyroidism to heart impairment remain poorly understood. Long non-coding RNAs (lncRNAs) have emerged as critical regulators of gene expression and may influence various pathophysiological processes.

Materials and methods: This study investigated the role of hub genes and lncRNAs in heart tissue under hypothyroid conditions through *in silico* analysis and experimental validation. Thirty mice were divided into five groups: (1) hypothyroid mice induced by daily injections of 8 mg/kg propylthiouracil (PTU) for one month (Hypo-Thy group); (2) hypothyroid mice treated with 0.4 mg/kg Selected phenolic Compounds of Hydroalcoholic extract of the aerial parts of *Dorema aucheri* (Herbal group); (3) hypothyroid mice undergoing endurance training (Ex group); (4) hypothyroid mice receiving both *Dorema aucheri* extract and endurance training (Ex+Herbal group); and (5) control mice. The expression levels of mRNAs and lncRNAs were assessed using real-time qPCR, and heart and thyroid functions were evaluated through ELISA methods.

Results: Data indicated a significant enhancement in the *Sirt-1/Nrf2/IL-6/Myh7/Col1a2* signaling axis in the Ex+ Herbal group compared to other groups. The expression levels of lncRNAs, including *HOTAIR*, *GAS5*, and *H19*, were notably altered in hypothyroid conditions.

Conclusion: Our findings suggest that endurance training and Selected Phenolic Compounds extract may effectively modulate lncRNA expression, offering potential avenues for addressing cardiac dysfunction associated with hypothyroidism. This study underscores the importance of exploring integrative approaches to mitigate heart dysfunction linked to thyroid hormone imbalances.

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Introduction

Low levels of thyroid hormones, in conjunction with elevated levels of thyroid-stimulating hormone (TSH), serve as critical diagnostic criteria for hypothyroidism. This hormonal imbalance indicates a reduced production of thyroid hormones, which can lead to a range of physiological disturbances. The measurement of TSH is particularly significant as it reflects the pituitary gland's response to low thyroid hormone levels, signaling the need for further investigation and potential intervention. Accurate diagnosis is essential for the effective management of hypothyroidism as it can significantly impact metabolic processes, cardiovascular health, and overall well-being. (Gao et al. 2015).

On the other hand, a previous study reported that endothelium-dependent vasodilatation is impaired in hypothyroidism status (Cai et al. 2015). Pathophysiological conditions frequently arise from the interplay of genetic, epigenetic, and environmental factors, which can be further modulated by a range of regulatory influences (Abedpoor et al. 2022; Hajibabaei et al. 2022). Long non-coding RNAs (lncRNAs) are distinguished by their capacity to regulate gene expression through various mechanisms, predominantly epigenetic in nature. These regulatory processes often involve competition with other endogenous RNAs (Mangraviti and De Windt 2022).

Phenolic compounds are considered beneficial and novel strategies to treat or manage various diseases (Lutz et al. 2019; Safavi et al. 2025). Based on the evidence, *Dorema aucheri* is enriched with phenolic compounds that could be implemented as alternative and complementary medicine in alleviating illness and preventing hallmarks synchronized with ordained medicine (Hossiniyan et al. 2021). A recent study showed that *Dorema aucheri* contains flavonoids, and its hydroalcoholic extract can be therapeutic for thyroid function with a specific dose (Aleebrahim-Dehkordy et

al. 2018). Flavonoids can reduce thyroid levels through their thyroperoxidase ability. In addition, phenolic compounds inhibit the activation of deiodinase type 1 which is activated explicitly by TSH (Pistollato et al. 2019). Coumarin is another compound of the *Dorema aucheri* that affects thyroid function by being able to convert T4 to T3 (Aleebrahim-Dehkordy et al. 2018). Studies have shown that coumarin derivatives have an increasing effect on thyroid hormones.

On the other hand, regular exercise training, especially endurance training, as a non-pharmacological intervention and with fewer side effects intervention can modulate proteins' role in molecular system biology, regulate metabolism, respond to oxidative stress, trigger antioxidant balance, and improve fitness performance and antioxidant capacity (Angadi et al. 2015). Hence, in this study, we addressed the following hypotheses:

1. Phenolic components of *Dorema aucheri* could offset hypothyroidism complications, cardiac dysfunction, and inflammation pathways by interacting with IL-6 protein as a druggable cut point in gene/lncRNAs network pathomechanism.
2. Endurance exercise could ameliorate cardiac dysfunction induced by hypothyroidism complications.
3. Combinational complementary medicine strategies consisting of regular aerobic exercise and phenolic components of *Dorema aucheri* extract could synergistically affect the hypothyroidism complications hallmarks.

Materials and Methods

Modeling and experimental animals

In this study, 30 C57BL/6 mice were provided by the Royan Institute. The mice were transferred to the animal house of the Isfahan (Khorasgan) Branch of Islamic Azad University. Mice were housed under standard conditions, including a 12 hr dark/12 hr light cycle, $24 \pm 3^{\circ}\text{C}$, and a humidity of $65\% \pm 5$. It should be noted that

this research was approved and conducted according to the laboratory animals via the ethics committee of Islamic Azad University, Khorasgan Branch (IR.IAU.KHUISF.REC.1400.190). After acclimation, the mice, which were 6 weeks old and weighed 20 ± 2.5 g, were randomized into five groups (n = 6):

1. Healthy mice (Control group).
2. Hypothyroid mice were induced by daily injection of 8 mg/kg PTU (Singh et al. 2020) (propylthiouracil) for one month (Hypo-Thy group).
3. Hypothyroid mice were treated with *Dorema aucheri* extraction (Herbal group).
4. Hypothyroid mice underwent endurance training (Ex group).
5. Hypothyroid mice treated with *Dorema aucheri* extract along with endurance training (Ex+ Herbal group).

After the last interventions, mice were euthanized under xylazine (10 mg/kg) and ketamine (80 mg/kg). Moreover, heart tissue and serum were separated and stored for further experimentation at -80°C . The cross-sectional left ventricular samples were fixed in 10% buffered formalin and decalcified using Ethylenediaminetetraacetic acid (EDTA) and Hydrochloric acid (HCL) for Hematoxylin & Eosin (H&E) staining.

Computational system methods in molecular biology

We screened the closest Gene Expression Omnibus (GEO) microarray dataset to detect significant genes with differential expression in mice hearts with pathological and physiological heart failure based on microarray profiling. Moreover, we used the R programming language software to analyze a microarray dataset with the limma package and RMA normalization methods. We display the results as a heatmap visualization by considering the differential expression score was statistically significant ($p < 0.05$). A logFC cut-off of ± 1 was used to identify

significantly up-regulated and down-regulated genes in this study.

To recognize the comprehensive protein-protein interaction network of common hub genes implicated in heart failure and hypothyroidism, we constructed a genetic network in STRING version 11.5 based on evidence and medium confidence (0.4) (Szklarczyk et al. 2021). Based on network parameters such as degree: 4, betweenness centrality: 0.01, and closeness centrality: 0.137, we obtained 62 nodes as hub genes in the hypothyroidism status. Using Gephi 0.9.2 software, we visualized the network's properties by setting the network parameters, eigenvector centrality, and modularity class and we established the hub genes network based on betweenness centrality and modularity class.

Prediction of lncRNAs: hub genes interaction based on computational approaches

This study aims to evaluate the involvement of lncRNAs in the pathophysiology of cardiac malfunction associated with hypothyroidism since lncRNAs are potential genetic molecules in regulating many biological processes such as transcription, gene expression, mRNA stability, and the competing endogenous RNA system (ceRNAs). The LncTarD and LncRNA Disease 2.0's algorithms allowed us to predict lncRNAs related to hub genes, diseases, and signaling pathways, particularly *IL-6* as the vital cut point in the pathogenesis network of hypothyroidism status and hypothyroidism complications on cardiac function. We next designed a network that would interconnect the prioritized hub genes with the expected microRNAs and lncRNAs.

Dorema aucheri medicinal chemistry compounds virtual screening

Based on system biology analysis, we described the *IL-6* node with the highest degree and betweenness centrality as a possible cut-point protein in the PPI network. Hence, we supposed that

hypothyroidism and heart failure might profit from a novel therapy strategy considering the druggable protein IL-6. To compute the binding affinity of effective compounds of *Dorema aucheri* over the IL-6 protein, the X-ray crystallography of the IL-6 protein was selected from the Protein Data Bank server (ID: 1ALU). Chimera 1.8.1 software was employed for protein structure preparation and optimization, removing ligands, non-complex ions, solvents, and extra chains. *Dorema aucheri* is a beneficial medicinal plant for treating diabetes, lowering cholesterol naturally, and controlling thyroid hormones, according to relevant literature. As a result, we performed the molecular docking to identify the suitable binding affinity of unique bioactive components in the *Dorema aucheri* extract on the druggable cut point protein. Therefore, we obtained 3D structures of bioactive components present in *Dorema aucheri* in the SDF format in the PubChem database. Then, using the Open Babel tool, we created a library containing 3D structures of bioactive compounds for molecular docking computations. The Vina search space was established in PyRx software for the molecular docking assay as $x=45.9496$, $y=45.3050$, and $z=53.4397$. In addition, we set binding affinity scores < -5 kcal/mol and Root Mean Square Deviation (RMSD) < 2 thresholds in this chemoinformatic investigation as significant bioactive compounds. RMSD value is a quality measure of stability between protein structures and ligands. RMSD < 2 is approved as a suitable cut-off value of the binding position in a molecular docking study.

Inducing hypothyroid mice

We found that PTU 8 mg/kg could be the optimal and efficient dose to induce hypothyroid mice. We injected PTU 8 mg/kg (intraperitoneally) for one month (Singh et al. 2020).

The alcoholic extraction method of aerial parts of *Dorema aucheri*

Dorema aucheri was harvested in the late spring in Iran's southern and western provinces, especially the Zagros mountains. A plant enriched with phenolic compounds has antioxidant and anti-inflammatory properties (Mianabadi et al. 2015). Aerial parts of the *Dorema aucheri* plant from the Kermanshah Zagros hills and mountains in early spring (2022) were harvested. Aerial parts were powdered using an electric mill after drying in the shade. Aerial parts were powdered using an electric mill after drying in the shade. The preparation of alcoholic extraction was operated by maceration technique in 70% ethanol and 30% water for 72 hr at room temperature. A Whatman No. 1 filter paper was employed to purify the liquid. The supernatant was evaporated after centrifugation (3000 rpm, 20 min) at room temperature. The extract was air-dried and stored at 4°C until use (Rasouli Vani et al. 2019). In this study, we administered *Dorema aucheri* extract by gavage at a 0.4 mg/kg dose for two months/five days a week (Mostafavi et al. 2013).

Endurance training protocol

The intensity of endurance training was moderate to high. The speed and intensity gradually increased to $\sim 80\%$ VO₂ max. The mice were exercised for 45 min at a slope of 0°. The exercise protocol was designed to progress from 10 m/min and 20% VO₂ max to 25 m/min and 80% VO₂ max (Abedpoor et al. 2018; Bahadorani et al. 2019; Rahimi et al. 2021).

Biochemical assay

After the last intervention, blood samples were centrifuged (3100 g, 5 min, 4°C) to separate the serum. We evaluated the concentration levels of ANP (Atrial Natriuretic Peptide, NBP2-66733), Deiodinase Iodothyronine Type II (DIO2, MBS014299), TSH (Thyroid-stimulating hormone, MBS455232), T3

Phenolic & exercise modulated mRNA-lncRNAs

(Triiodothyronine, MBS262762), and T4 (Thyroxine, MBS700665).

qPCR-Real time

Total RNA was isolated from cardiac tissue utilizing TRIzol reagent in accordance with the manufacturer's instructions. The amount and integrity of RNA were evaluated with a NanoDrop spectrophotometer. Complementary DNA

(cDNA) was produced utilizing the TaKaRa cDNA synthesis kit in accordance with the manufacturer's guidelines. Quantitative real-time PCR was conducted with SYBR Green chemistry on a Rotor-Gene 6000 apparatus. 18S rRNA served as the internal reference gene for the standardization of mRNA and lncRNA expression levels assessment. Primer sequences are shown in Table 1.

Table 1. Primers sequences for qRT-PCR.

Gene Symbol	Accession No.	Primer Sequence	Product Size (bp)
18S rRNA	NR_003278.1	CGGACACGGACAGGATTG	85
		TCGCTCCACCAACTAAGAAC	
IL-6	NM_031168	CCTAGTGCGTTATGCCTAAG	131
		AGTGTCCCAACATTCATATTG	
Myh7	NM_080728.2	TGCAAAGGCTCCAGGTCTGAGGGC	203
		GCCAAACACCAACCTGTCCAAGTTC	
Col1a2	NM_007743	TTCTGTGGGTCTCTGCTGGGAAA	123
		TTGTCACCTCGGATGCCTTGAG	
Sirt1	NM_019812	GGCAGTAACAGTGACAGT	110
		CTCTCCGTATCATCTTCCAA	
Nrf2	NM_010902.4	GCTCTCCATATTCCATTCC	130
		TACCTCTCCTGCGTATATC	
Hotair	NR_047528.1	TTGCTCTCCTTATCATCC	132
		ATTAGTGCCTCTCAGTCC	
Gas5	NR_166055.1	AGTGTGGACCTCTGTGAT	78
		CTCGTAAGCCTTCATCCTCCT	
H19	NR_130974.1	TACCCCGGGATGACTTCATC	186
		TATCTCCGGGACTCCAAACC	

Hematoxylin and Eosin staining

The mice were sacrificed immediately following the final intervention, and the hearts were removed for histological and pathological examination. After the last interventions, the mice were fasted for 6 hours and euthanized under the combined injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Heart tissue was collected and snap-frozen in Liquid nitrogen. In addition, blood samples were

collected, and the serums were separated. Heart tissue and serum samples were transferred to the medical genetics laboratory for storage at -80°C.

The samples were decalcified with EDTA and HCl and then fixed in 10% buffered formalin for this investigation (Richard-Allan Scientific, Kalamazoo, MI, USA). The cardiac tissue samples were stained with Hematoxylin and Eosin (H&E).

Statistical analysis

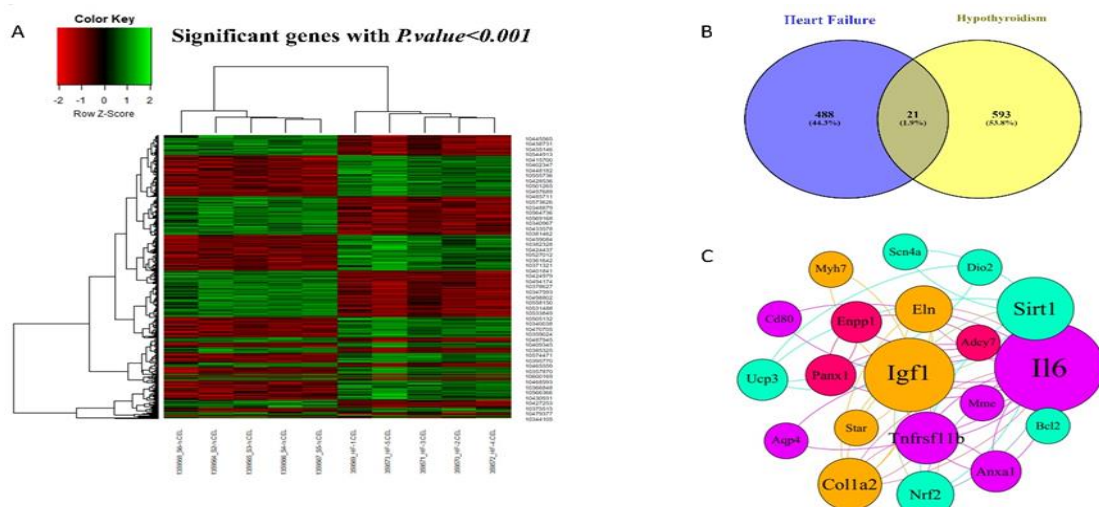
The results were calculated with GraphPad Prism Software (Version 9, GraphPad Software Inc., La Jolla, CA). The Shapiro-Wilk test was used to assess the normal distribution. Furthermore, a one-way analysis of variance (ANOVA) with Tukey's post hoc analysis was used to analyze all data. Results are indicated as mean±standard deviation (SD), and differences at $p<0.05$ demonstrated significance in all analyses.

Results

Computational systems biology-driven differential pathway expression

Microarray profiling analysis in mice hearts with pathological and physiological cardiac hypertrophy revealed that among 6922 significant genes with differential expression patterns in heart failure conditions compared to the sham group, 383 proteins were up-regulated, and 140 nodes were down-regulated by applying $\log FC \pm 1$. Hence, the highest significant differential expression of genes could be a candidate strategy for controlling and treating heart dysfunction pathogenesis induced by hypothyroidism. Consequently, the heatmap graphical representation demonstrates significant differential

expression of genes in heart failure status vs. sham ($p<0.001$) (Figure 1A). Using the Venn diagram tool, we suggest that hypothyroidism and heart failure could have a cross-talk via the involvement of 21 common components, including *UCP3*, *MME*, *AQP4*, *SCN4A*, *DIO2*, *IL-6*, *MYH7*, *STAR*, *IGF1*, *BCL2*, *CD80*, *ENPP1*, *ANXA1*, *ADCY7*, *COL1A2*, *ELN*, *TNFRSF11B*, *ATP8B1*, *PANX1*, *NRF2*, and *SIRT1* (Figure 1B). Hence, constructing a genetic network including 21 common genes based on medium confidence in *Mus musculus* species shows that IL-6, with the highest degree and highest betweenness centrality, could be pointed out as a druggable candidate in heart dysfunction induced by hypothyroidism status (Figure 1C). Enrichment analysis and literature mining based on KOBAS-i and Erich-r algorithms pointed out that these hub genes, EGFR tyrosine kinase inhibitor resistance, AGE-RAGE, P53, HIF-1, endocrine resistance, platelet activation, apoptosis, hypertrophic cardiomyopathy, dilated cardiomyopathy, Cushing syndrome, hypothyroidism, diabetes mellitus, heart failure, hypertensive diseases, left ventricular hypertrophy might be associated with heart pathogenesis induced by hypothyroidism (Figure 1D and 1E).



Phenolic & exercise modulated mRNA-lncRNAs

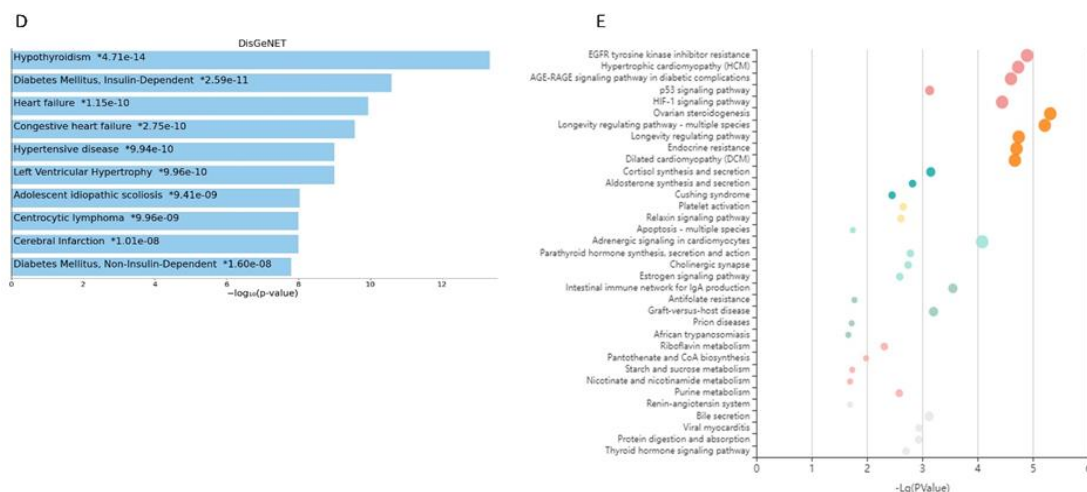


Figure 1. System biology and Enrichment analysis. A: The heatmap diagram shows the differentially expressed genes between heart failure status vs. sham group ($P < 0.01$). B: There are 21 intersectional genes between hypothyroidism and significant genes involved in heart failure, including *UCP3*, *MME*, *AQP4*, *SCN4A*, *DIO2*, *IL-6*, *MYH7*, *STAR*, *IGF1*, *BCL2*, *CD80*, *ENPP1*, *ANXA1*, *ADCY7*, *COL1A2*, *ELN*, *TNFRSF11B*, *ATP8B1*, *PANX1*, *NRF2*, and *SIRT1*. C: A network of protein-protein interactions was constructed, including common hub genes involved in hypothyroidism and heart failure. Modularity classification of hub genes shows that the isochromatic genes are involved in the common signaling pathways and functions. D and E: The following common molecular signaling pathways were identified by browsing hub genes in enrichment databases: EGFR tyrosine kinase inhibitor resistance; AGE-RAGE; P53; HIF-1; endocrine resistance; platelet activation; apoptosis; hypertrophic cardiomyopathy; dilated cardiomyopathy; Cushing syndrome; hypothyroidism; diabetes mellitus; heart failure; hypertensive diseases; left ventricular hypertrophy.

lncRNA signatures underlying hypothyroidism-associated cardiac dysfunction

HOTAIR, *GAS5*, and *H19* were predicted as regulatory factors through various biological processes targeting the *IL-6* gene after browsing lncRNAs prediction databases based on genes, cardiomyopathy diseases, and molecular signaling pathways. Based on annotation results, we found that lncRNA *HOTAIR* could positively modulate *IL-6* in DNA damage, senescence, and NF- κ B signaling pathways. Moreover, evidence shows that *H19* interacted with ceRNA *let-7: IL-6* in the inflammation response of abdominal aortic aneurysms. Further, *GAS5*, with a sponge mechanism, targets miR-26a and positively modulates the apoptosis process in atherosclerosis conditions. Hence, we designed a network of proteins/genes:lncRNAs: microRNAs interactions based on the miRNet algorithm (Figure 2A). Enrichment of this network

has shown that cardiac muscle contraction, hypertrophic cardiomyopathy, legionellosis, amphetamine addiction, dilated cardiomyopathy, ECM receptor interaction, Toll-like receptor signaling pathway, and cytokine-cytokine receptor interaction are associated with this network. Moreover, in this network, we found that genes and lncRNAs interacted through microRNAs.

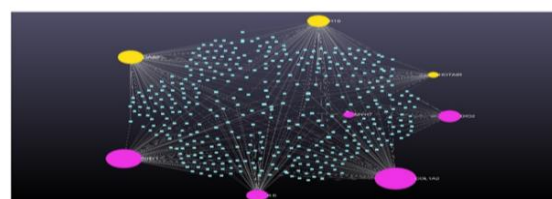


Figure 2. A: Non-coding RNAs network. The comprehensive network displayed genetic interactions between common hub genes, lncRNAs, and microRNAs. The comprehensive network displayed genetic interactions between common hub genes, lncRNAs, and microRNAs. This network is associated with cardiac muscle contraction, hypertrophic cardiomyopathy, legionellosis, amphetamine addiction, dilated cardiomyopathy, ECM receptor interaction, Toll-like receptor signaling pathway, and cytokine-cytokine receptor interaction.

Virtual screening of medicinal chemistry

We found 6 phenolic compounds in the aerial parts of the *Dorema aucheri* plant consisting of coumarin, galic acid, curzerene, coumaric acid, caffeic acid, and spathulenol. Virtual screening of phenolic compounds by the molecular docking method showed that these phenolic substances, with an acceptable binding affinity score, bound to the IL-6 active site (Figure 3A-F and Table 2). The highest

affinity score was curzerene and caffeic acid (-6.0 kcal/mol and RMSD<2). Further, coumarin, with the lowest binding affinity score (-5.1 kcal/mol and RMSD<2), is bound to the IL-6 active site. Hence, we suggest that phenolic compounds in the aerial parts of the *Dorema aucheri* plant might offset cardiac dysfunction induced by hypothyroidism conditions through IL-6 druggable cut-point and PPIs network.

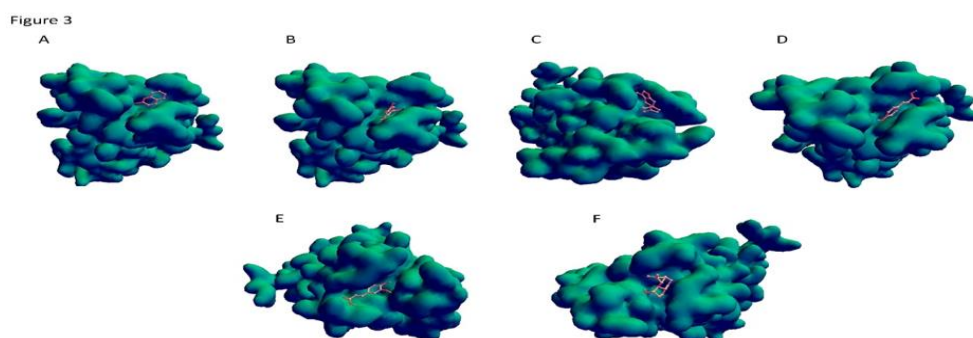


Figure 3. Virtual screening of *Dorema aucheri* extract's phenolic compounds on the IL-6 protein as effective cut-point in hypothyroidism complication and heart dysfunction induced by hypothyroidism. A-F: Molecular docking computing estimated phenolic compounds including coumarin, galic acid, curzerene, coumaric acid, caffeic acid, and spathulenol on IL-6 protein with suitable binding affinity score (<-5 kcal/mol).

Table 2. Virtual screening of phenolic compounds of aerial parts of the *Dorema aucheri* on the active site of the IL-6 protein.

	Control	Hypo-Thy	Herbal	EX	Ex-Herbal	p value
T3 (ng/mL)	0.65±0.05	0.15±0.02 ^a	0.39±0.03 ^{ab}	0.43±0.024 ^{ab}	0.67±0.031 ^{bcd}	p< .05
T4 (ng/mL)	8±1.5	3.4±1.3 ^a	5.3±0.9 ^{ab}	5.67±1.5 ^{ab}	7.43±0.3 ^{bcd}	p< .05
TSH (pg/mL)	6±0.34	16.3±0.5 ^a	12.3±0.04 ^{ab}	11.4±0.2 ^{ab}	9.34±0.39 ^{bcd}	p< .05

Dysregulated cardiac–thyroid axis in hypothyroid mice

In Table 3, we indicate that the serum concentration of T3 and T4 decreased in the Hypo-Thy group compared with the Control. Moreover, the hypothyroid mice's TSH serum concentrations significantly increased compared with the Control (Table 3). In this study, to evaluate heart function, the concentration of Dio2 and ANP was assessed by the ELISA method. We found that the Dio2 and ANP concentration of the Hypo-Thy group was enhanced compared with the Control (Figure 4A and B). On the other hand, the relative expression of the *IL-6/Myh7/Col1a2* in cardiac tissue of the

Hypo-Thy group significantly increased compared with the Control (Figure 4 C-D). In addition, the expression level of *Sirt-1/Nrf2* was significantly reduced in the cardiac tissue of hypothyroidism mice compared with control mice (Figures F and G). Based on these data, we found that the hypothyroid mice's heart and thyroid function were dysregulated.

Molecular and protein normalization by endurance training and *Dorema aucheri*

We demonstrated that the T3, T4, TSH, Dio2, and ANP serum concentrations significantly improved in hypothyroid mice with endurance training (ExT group).

Furthermore, we indicated that T3, T4, and TSH serum concentrations were regulated by consuming *Dorema aucheri* extract in hypothyroid mice (Table 3). Moreover, the Dio2 and ANP serum concentrations were improved by consuming *Dorema aucheri* extract in hypothyroid mice (Figure 4A and B). Notably, the endurance training and *Dorema aucheri* extract interaction was effective compared with other groups. Based on these data, endurance training and *Dorema aucheri* extract ameliorated T3, T4, and TSH serum concentrations in hypothyroid conditions (Table 3). Moreover, the hypothyroid mice underwent endurance training (ExT group), and the *IL-6/Myh7/Col1a2* expression level decreased compared with the Hypo-Thy group

(Figures 4 C-E). Also, consumption of the *Dorema aucheri* extract declined the *IL-6/Myh7/Col1a2* level. In this study, we revealed that the interactions of the endurance training and *Dorema aucheri* extract significantly reduced the relative expression of the *IL-6/Myh7/Col1a2* compared with the other groups. Interestingly, we found that the expression levels of *Sirt-1/Nrf2* were remarkably increased by endurance training and *Dorema aucheri* extract compared with the Hypo-Thy group (Figures 4 F and G). Hence, endurance training and *Dorema aucheri* extract reversed the molecules and proteins involved in heart and thyroid dysfunction.

Table 3. The concentration of the T3, T4, and TSH

PubChem ID	phenolic Compound Name	Binding affinity (kcal/mol)	RMSD
323	Coumarin	-5.1	<2
370	Galic acid	-5.5	<2
572766	Curzerene	-6.0	<2
637542	Coumaric acid	-5.8	<2
689043	Caffeic acid	-6.0	<2
92231	Spathulenol	-5.8	<2

a: compared to the Control, b: compared to the Hypo-Thy, c: compared Herbal, d: compared to EX.

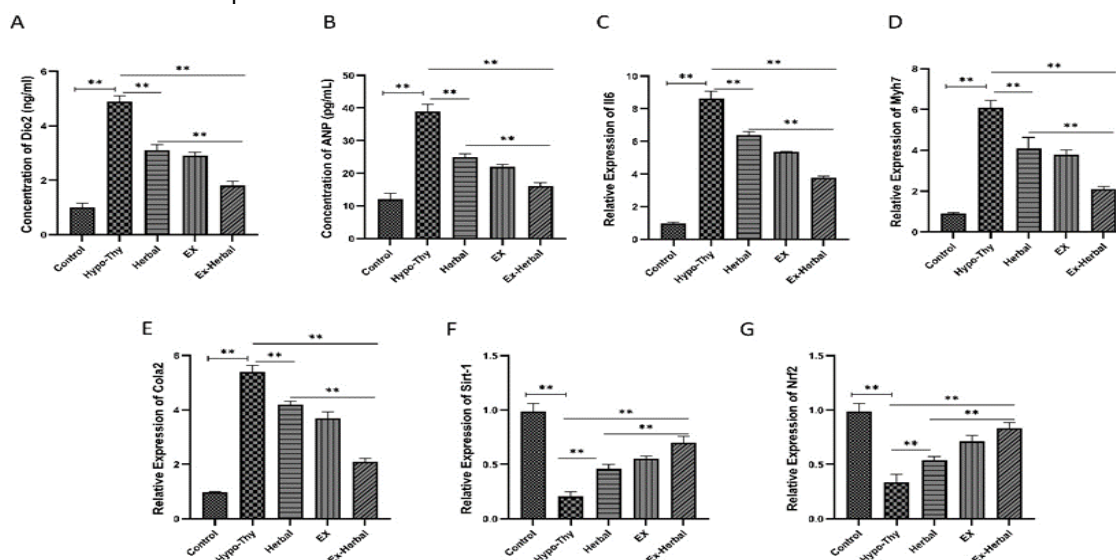


Figure 4. Endurance training and *Dorema aucheri* regulated biochemical and mRNAs network in hypothyroid mice. A. Concentration of Deiodinase Iodothyronine Type II (Dio2) in heart tissue, B. Concentration of Atrial Natriuretic Peptide (ANP) in heart tissue, C-G. Relative messenger RNA (mRNA) expression levels in heart tissue, specifically: C. Interleukin 6 (*IL-6*), D. Myosin Heavy Chain 7 (*Myh7*), E. Collagen alpha-2(I) chain (*COL1A2*), F. Sirtuin 1 (*Sirt-1*), and G. Nuclear factor erythroid 2-related factor 2 (*Nrf2*). All data are presented as mean ± standard deviation (SD) for n = 6 biological replicates per group. p-value less than 0.05 ($p < 0.05$) was considered statistically significant. (mean ± SD; n = 6, $p < 0.05$).

Endurance training and *Dorema aucheri* improved the pathological features

The pathological feature indicated necrotic areas in the Hypo-Thy group compared with the Control group (Figure 5). Our data indicated that the necrosis areas and cell death with the loss of the nuclei significantly decreased by endurance training and *Dorema aucheri* extract. Moreover, we found that endurance training and *Dorema aucheri* extract were effective compared with other groups. Notably, the morphology of the heart tissue treated with endurance training and *Dorema aucheri* improved compared with the Hypo-Thy group (Figure 5). Based on Figure 5, we indicated cardiac tissue failure, including cytoplasmic degeneration, congested vessels, loss of striation, congestion, edema, and lymphocytic infiltration in the Hypo-Thy group. In the Ex group and Herbal group, relative striation and cytoplasmic regeneration were found.

Reprogramming of cardiac systems biology networks in response to combined endurance training and *Dorema aucheri*

Based on the *in silico* analysis, we found that the *IL-6* molecule had a critical role in heart failure and hypothyroidism. Our analysis found that *HOTAIR*, *GAS5*, and *H19* could target *IL-6*. We evaluated the expression level of the *HOTAIR*, *GAS5*, and *H19* in hypothyroidism conditions. Our data indicated that the expression level of *HOTAIR*, *GAS5*, and *H19* increased in the Hypo-Thy group compared with the Control group (Figures 6 A-C). Moreover, we found that endurance training and *Dorema aucheri* extract could regulate the expression level of these non-coding RNAs targeted by *IL-6* (Figures 6 A-C).

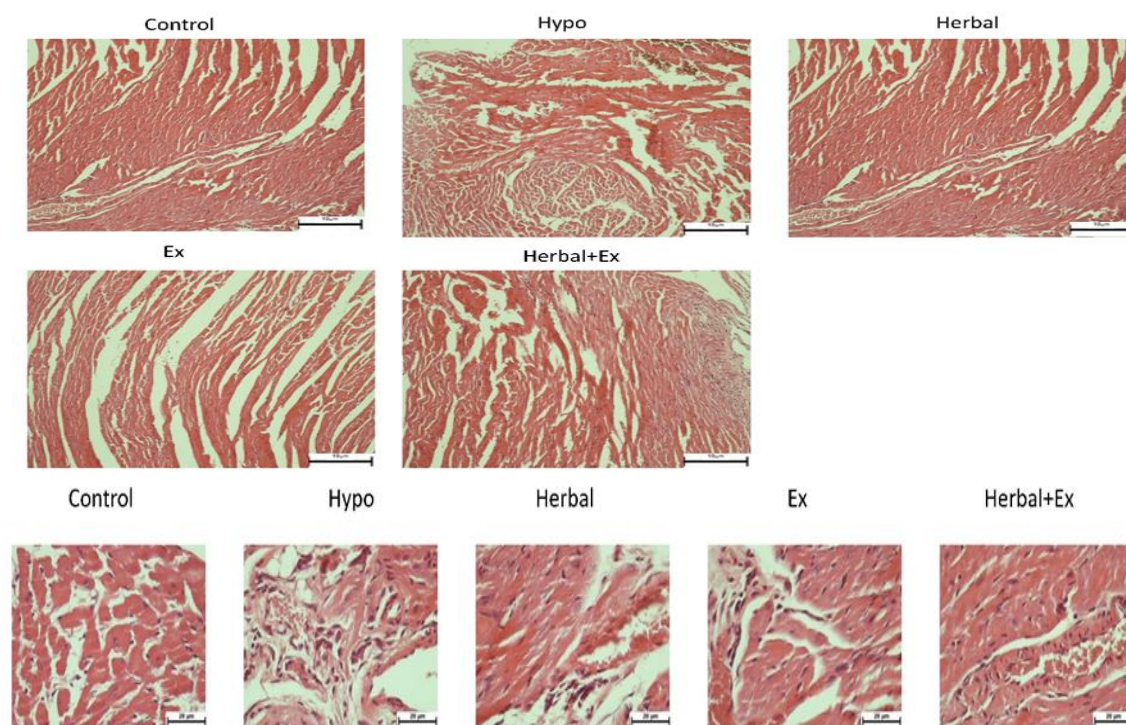


Figure 5. Endurance training and *Dorema aucheri* in Hypothyroidism mice improved the Pathological feature. Representative slices of left ventricular heart tissue from the experimental groups, stained with hematoxylin and eosin. Sections were analyzed at 200x and 400x magnification. The hypothyroid cohort had significant cardiac damage, characterized by necrosis, nuclear loss, striational loss, congestion, edema, cytoplasmic degeneration, and inflammatory infiltration. Conversely, endurance training, *Dorema aucheri* extract, and particularly the combined intervention enhanced cardiac architecture and diminished histological damage. Arrows denote necrotic myocytes and regions of structural disturbance. Scale bars: 40 μ m.

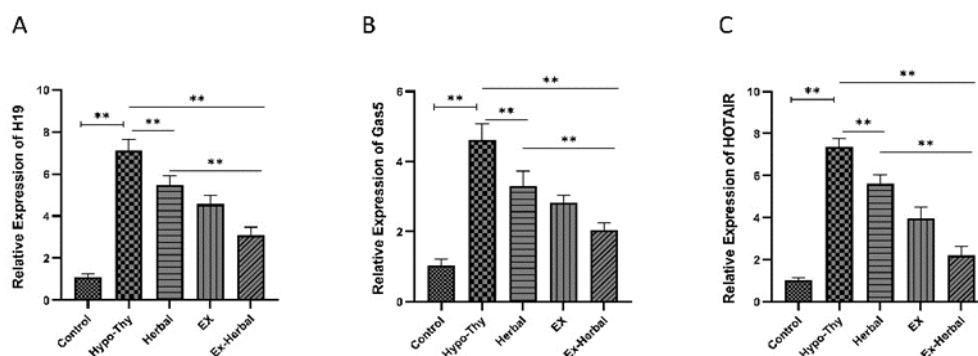


Figure 6: Endurance training and *Dorema aucheri* modulated the lncRNAs network in the heart of hypothyroid mice. A-C: The level expression of *H19*, *GAS5*, and *HOTAIR*. (mean $\pm$ SD; n = 6, $p < .05$).

Discussion

Our findings indicate that endurance training, along with the administration of *Dorema aucheri* extract, modulates the *Sirt1/Nrf2/IL-6/Myh7/Col1a2* signaling pathway in the cardiac tissue of hypothyroid mice. Furthermore, *in silico* analyses revealed that the long non-coding RNAs *HOTAIR*, *GAS5*, and *H19* can target the *IL-6* gene. Additionally, we observed a dysregulation in the expression levels of *HOTAIR*, *GAS5*, and *H19* in the hearts of hypothyroid mice. Notably, our data demonstrate that both endurance training and *Dorema aucheri* extract significantly enhance the expression of *HOTAIR*, *GAS5*, and *H19*. This study shows that endurance exercise alongside *Dorema aucheri* extract enhances thyroid function, cardiac biochemical markers, histopathological damage, and the expression of specific cardiac genes and lncRNAs in hypothyroid mice. The observed effects were associated with the modification of the *Sirt1/Nrf2/IL-6/Myh7/Col1a2* axis, indicating that both oxidative stress-related and inflammatory pathways are involved in the cardiac response to hypothyroidism and its reversal with the combined intervention. Tang *et al.* indicated that hypothyroidism results in significant cardiac structural and functional impairments, reinforcing the notion that thyroid shortage directly causes myocardial damage. This study demonstrated diminished T3 and T4 levels, elevated TSH,

and significant histological degradation in the hypothyroid group, thus demonstrating the effective induction of the illness model and the cardiac repercussions of thyroid hormone shortage. This study reveals a significant downregulation of *IL-6*, *Myh7*, and *Col1a2*, accompanied by the restoration of *Sirt1* and *Nrf2* expression after endurance exercise and *Dorema aucheri* treatment. This pattern indicates a decrease in inflammatory signaling, decreased extracellular matrix remodeling, and enhanced redox equilibrium in cardiac tissue. Given that *IL-6* is a pivotal modulator of inflammation and cardiac remodeling, its regulation may serve as a crucial mechanism by which the therapies manifest their effects. The integrated intervention seemed to elicit the most robust response, suggesting that exercise and phytochemical supplementation may function synergistically. Growing evidence has demonstrated that hypothyroidism could trigger a reduction in heart rhythm and contraction dysregulation that leads to an increase in systemic vascular resistance, an increase in afterload, and a decrease in stroke volume and cardiac output in the cardiovascular system (Bengel *et al.* 2000; Schmidt-Ott and Ascheim 2006). The lncRNA discoveries offer further mechanistic understanding. *HOTAIR*, *GAS5*, and *H19* exhibited dysregulation in hypothyroid hearts, which was somewhat rectified by the therapies. These lncRNAs have been associated with inflammatory

signaling, cardiac remodeling, and post-transcriptional regulation in various clinical contexts. Consequently, their modified expression in this study indicates that hypothyroidism-related cardiac dysfunction may engage lncRNA-mediated gene regulation networks, especially those associated with *IL-6*. While these data are associative, they indicate a possible regulatory function of lncRNAs in the etiology and recovery of thyroid-associated heart damage. This research possesses multiple limitations. The results were derived from a murine model, and direct application to humans should be approached with caution. Secondly, the sample size was moderate, however adequate to identify significant variations among groups in the current design. Third, we did not do electrocardiogram (ECG) analysis or direct hemodynamic measurements, which would have yielded a more thorough evaluation of heart function. Fourth, mechanistic validation utilizing loss- or gain-of-function methodologies, such as siRNA-mediated silencing, was not conducted. The study failed to explore dose-response relationships for *Dorema aucheri* thoroughly analyze varying exercise intensities and durations. Ultimately, although the data support a protective function of endurance training and *Dorema aucheri*, the specific biochemical intermediates connecting both interventions to lncRNA regulation have yet to be identified. The current findings suggest that endurance exercise and *Dorema aucheri* extract enhance thyroid and heart dysfunctions in hypothyroid mice, correlating with the modulation of inflammatory, oxidative stress, and lncRNA-related pathways. These findings endorse the viability of integrated lifestyle and phytochemical approaches as a foundation for forthcoming mechanistic and translational research in hypothyroidism-associated heart dysfunction (Abedpoor et al. 2024).

In this study, we identified 21 shared components between hypothyroidism and

heart failure that exhibited significant differential expression in cardiac tissue, playing a critical role in the hallmark features and complications associated with hypothyroidism. Furthermore, we elucidated the key molecular signaling pathways involving *UCP3*, *MME*, *AQP4*, *SCN4A*, *DIO2*, *IL-6*, *MYH7*, *STAR*, *IGF1*, *BCL2*, *CD80*, *ENPP1*, *ANXA1*, *ADCY7*, *COL1A2*, *ELN*, *TNFRSF11B*, *ATP8B1*, *PANX*, *NRF2*, and *SIRT1*. Consequently, our bioinformatics and enrichment analysis suggest potential links between hub genes and resistance to EGFR tyrosine kinase inhibitors, as well as associations with pathways related to AGE-RAGE, P53, HIF-1, endocrine resistance, platelet activation, apoptosis, hypertrophic cardiomyopathy, dilated cardiomyopathy, Cushing's syndrome, hypothyroidism, diabetes mellitus, heart failure, hypertensive diseases, and left ventricular hypertrophy. These factors may collectively contribute to the pathogenesis of heart disease induced by hypothyroidism. Prior studies focused on cardiovascular diseases have underscored the protective roles of sirtuins in promoting cardiac survival and enhancing tolerance to oxidative stress (Meng et al. 2017); specifically, the role of SIRT1 in safeguarding the heart has been highlighted in studies involving SIRT1-knockout mice, where evidence of exacerbated myocardial injury following ischemia/reperfusion events has been demonstrated (Wang et al. 2018).

Evidence indicates that high-intensity interval training is more effective than moderate-intensity continuous training in improving daytime systolic and diastolic blood pressure. Our findings suggest that both endurance training and the intake of *Dorema aucheri* can enhance cardiac and thyroid function in hypothyroid mice. Importantly, endurance training and *Dorema aucheri* consumption regulate the expression levels of the *Sirt-1/Nrf2/IL-6/Myh7/Col1a2* signaling axis in the hearts of hypothyroid mice. One study has demonstrated that hypothyroidism can lead

to significant progressive systolic dysfunction, characterized by ventricular dilation, impaired myocardial perfusion, and a reduction in myocardial arterioles; thus, thyroid dysfunction can independently contribute to cardiac failure (Tang et al. 2005). The modulation of the *Nrf2-Keap1* axis through regular exercise not only contributes to enhanced oxidative stress resistance but also supports overall metabolic health. These findings highlight the importance of physical activity in promoting cellular resilience and underscore its potential therapeutic implications for conditions associated with oxidative stress and inflammation (Rahimi et al. 2021).

On the other hand, the interaction between endurance training and consumption of *Dorema aucheri* had a greater effect on the heart and thyroid function in the hypothyroid mice. Moreover, the expression level of the Sirt-1/Nrf2/IL-6/Myh7/Col1a2 axis in the heart of hypothyroid mice was ameliorated by the interaction between endurance training and consumption of the *Dorema aucheri*. Evidence was reported that *Dorema aucheri* extract (Chemical composition and cytotoxic activity of the essential oil from the aerial parts of *Dorema aucheri*) has anti-hyperlipidemia, anti-hypercholesterolemia, antioxidant, anti-tumor, anti-diabetic, and anti-inflammatory properties and anti-microbial effects on numerous diseases (Hossiniyan et al. 2021).

Non-coding RNAs (ncRNAs) have emerged as significant post-transcriptional regulators in relation to disease progression, prognosis, diagnostics, and therapeutic approaches. A substantial body of evidence suggests that the expression profiles of long non-coding RNAs (lncRNAs) are closely linked to the pathological conditions associated with cardiac dysfunction and thyroid disorders (Chen et al. 2022). Liu et al. showed that lncRNA H19, by targeting miR-675, could control CaMKII δ function in cardiomyocytes (Liu et al. 2016). Liu and

colleagues revealed that the long non-coding RNA H19 regulates the function of CaMKII δ in cardiomyocytes by targeting miR-675. Moreover, Wang et al. demonstrated that H19 employs a competing endogenous RNA (ceRNA) mechanism to target the MicroRNA-145-3p/*SMAD4* axis, thereby inhibiting cardiac hypertrophy (Wang et al. 2022).

In the present study, we observed that the relative expression levels of specific Non-coding RNAs—*HOTAIR*, *GAS5*, and *H19*—were significantly dysregulated in the hearts of hypothyroid mice. Through experimental and bioinformatics analyses, we demonstrated that the expression levels of *HOTAIR*, *GAS5*, and *H19* are associated with heart dysfunction induced by hypothyroidism. Furthermore, our findings indicate that endurance training and *Dorema aucheri* extract can enhance the expression levels of *HOTAIR*, *GAS5*, and *H19*. This suggests that both endurance training and *Dorema aucheri* extract may regulate the expression of these non-coding RNAs. Abedpoor *et al.* emphasized that endurance training and supplementary consumption can influence the expression profiles of lncRNAs. They reported that the differential expression of lncRNAs induced by regular training and supplementation could modulate pathological networks and improve various disease hallmarks (Abedpoor et al. 2022). One of the limitations of this study is the absence of small interfering RNA (siRNA) interventions in the context of hypothyroidism. Additionally, we did not compare different doses of *Dorema aucheri* in our experiments. Furthermore, we were unable to measure electrocardiogram (ECG) parameters. The variations in intensity, duration, and repetition of aerobic exercise training were also not evaluated. Despite these limitations, our findings indicate that physical activity and the consumption of *Dorema aucheri* can regulate thyroid hormone levels and the associated signaling pathways involved in hypothyroidism. This

study suggests that future experimental investigations should focus on evaluating cardiac function and conducting clinical trials in accordance with established guidelines. As one of the limitations of this study, we did not use siRNA in the hypothyroidism condition. In addition, different doses of *Dorema aucheri* were not compared in this study. Moreover, we could not measure the electrocardiogram (ECG). Furthermore, the different intensity, duration, and repetition of aerobic exercise training were not evaluated. Based on this, we found that physical activity and consumption of *Dorema aucheri* could regulate the thyroid hormone and the signaling pathway involved in the hypothyroidism condition. This study suggested that the next experimental evaluated the heart function and assessed on the clinical trial according to the guidelines.

In conclusion, our study indicates that endurance training and *Dorema aucheri* extract have beneficial effects on the heart and thyroid function of hypothyroid mice. Our data suggest that these interventions can regulate the *Sirt-1/Nrf2/IL-6/Myh7/Col1a2* signaling axis and specific non-coding RNAs in the hearts of hypothyroid mice. Consequently, the oxidative stress and inflammatory status associated with hypothyroid-induced heart dysfunction may be improved through endurance training combined with the consumption of a safe dose of *Dorema aucheri* extract. Furthermore, we found that the *Sirt-1/Nrf2/IL-6/Myh7/Col1a2* axis can be modulated in the hearts of hypothyroid mice following this combined intervention. Notably, our findings indicate that the lncRNAs *HOTAIR*, *GAS5*, and *H19* can target the *IL-6* gene and influence its expression through post-transcriptional mechanisms. Additionally, we demonstrated that the expression levels of *HOTAIR*, *GAS5*, and *H19* were dysregulated in the hearts of hypothyroid mice, but endurance training and *Dorema*

aucheri extract significantly improved their expression levels.

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Conflicts of interest

The authors had no competing interests.

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Ethical Considerations

This study was performed and approved based on all the protocols of laboratory animals by the Ethics Committee of Islamic Azad University, Khorasgan Branch (IR.IAU.KHUISF.REC.1400.190).

Code of Ethics

IR.IAU.KHUISF.REC.1400.190

Authors' Contributions

Design of study was done by NT and FT. Experiments was performed by NT and FT. Analyses and data mining were performed by NT and FT. The manuscript was written by NT and FT.

Abbreviations

Thyroid-Stimulating Hormone (TSH), Ethylenediaminetetraacetic Acid (EDTA), Hydrochloric Acid (HCL), Hematoxylin & Eosin (H&E), Gene Expression Omnibus (GEO), Propylthiouracil (PTU), Complementary DNA (cDNA), Non-coding RNAs (ncRNAs), Competing Endogenous RNA (ceRNA), long non-coding RNAs (lncRNAs), electrocardiogram (ECG)

References

- Abedpoor N, Taghian F, Ghaedi K, et al. (2018) PPAR γ /Pgc-1 α -Fndc5 pathway up-regulation in gastrocnemius and heart muscle of exercised, branched chain amino acid diet fed mice. *Nutr. Metab.* 15(1):1-15

- Abedpoor N, Taghian F, Hajibabaie F (2022) Cross Brain–Gut Analysis Highlighted Hub Genes and LncRNA Networks Differentially Modified During Leucine Consumption and Endurance Exercise in Mice with Depression-Like Behaviors. *Mol. Neurobiol.*:1-18
- Abedpoor N, Taghian F, Jalali Dehkordi K, Safavi K (2024) Sparassis latifolia and exercise training as complementary medicine mitigated the 5-fluorouracil potent side effects in mice with colorectal cancer: bioinformatics approaches, novel monitoring pathological metrics, screening signatures, and innovative management tactic. *Cancer Cell Int* 24(1):141
- Aleebrahim-Dehkordy E, Ansari pour S, Rafieian-Kopaei M, Saberianpour S (2018) Effects of substances on plants' active compounds on changes in the hormone levels of the pituitary-thyroid axis in hyperthyroidism and hypothyroidism. *Pharmacogn Rev* 12(23)
- Angadi SS, Mookadam F, Lee CD, Tucker WJ, Haykowsky MJ, Gaesser GA (2015) High-intensity interval training vs. moderate-intensity continuous exercise training in heart failure with preserved ejection fraction: a pilot study. *Appl. Physiol.* 119(6):753-758
- Bahadorani M, Tavalae M, Abedpoor N, Ghaedi K, Nazem MN, Nasr-Esfahani MH (2019) Effects of branched-chain amino acid supplementation and/or aerobic exercise on mouse sperm quality and testosterone production. *Andrologia* 51(2):e13183
- Bengel FM, Nekolla SG, Ibrahim T, Weniger C, Ziegler SI, Schwaiger M (2000) Effect of thyroid hormones on cardiac function, geometry, and oxidative metabolism assessed noninvasively by positron emission tomography and magnetic resonance imaging. *J. Clin. Endocr.* 85(5):1822-1827
- Cai Y, Manio MM, Leung GP, Xu A, Tang EH, Vanhoutte PM (2015) Thyroid hormone affects both endothelial and vascular smooth muscle cells in rat arteries. *Eur. J. Pharmacol.* 747:18-28
- Chen F, Li W, Zhang D, et al. (2022) MALAT1 regulates hypertrophy of cardiomyocytes by modulating the miR-181a/HMGB2 pathway. *Eur. J. Histochem: EJH* 66(3)
- Gao C, Yang B, Guo Q, Wei L, Tian L (2015) High thyroid-stimulating hormone level is associated with the risk of developing atherosclerosis in subclinical hypothyroidism. *Horm Metab Res.* 47(03):220-224
- Hajibabaie F, Abedpoor N, Assareh N, Tabatabaiefar MA, Shariati L, Zarrabi A (2022) The importance of SNPs at miRNA binding sites as biomarkers of gastric and colorectal cancers: a systematic review. *J. Pers. Med.* 12(3):456
- Hossiniyan SA, Ghalamfarsa F, Rostami-Yasuj S, et al. (2021) Chemical composition and cytotoxic activity of the essential oil from the aerial parts of *Dorema aucheri*. *J Herbmed Pharmacol.* 10(3):344-350
- Liu L, An X, Li Z, et al. (2016) The H19 long noncoding RNA is a novel negative regulator of cardiomyocyte hypertrophy. *Cardiovasc. Res.* 111(1):56-65
- Lutz M, Fuentes E, Ávila F, Alarcón M, Palomo I (2019) Roles of phenolic compounds in the reduction of risk factors of cardiovascular diseases. *Molecules* 24(2):366
- Mangraviti N, De Windt LJ (2022) Long Non-Coding RNAs in Cardiac Hypertrophy. *Front. mol.* 2:836418
- Meng X, Tan J, Li M, Song S, Miao Y, Zhang Q (2017) Sirt1: role under the condition of ischemia/hypoxia. *Cell Molec Neurobiol.* 37(1):17-28
- Mianabadi M, Hoshani M, Salmanian S (2015) Antimicrobial and Anti-oxidative Effects of Methanolic Extract of *Dorema aucheri* Boiss. *JAST.* 17(3):623-634
- Mostafavi SH, Fazilati M, Mostafavi S-A, et al. (2013) Hepatotoxicity of *Dorema aucheri* (Bilhar) in albino mice. *AIM.* 16(9)
- Pistollato F, Masias M, Agudo P, Giampieri F, Battino M (2019) Effects of phytochemicals on thyroid function and their possible role in thyroid disease. *Ann N Y Acad Sci.* 1443(1):3-19
- Rahimi G, Heydari S, Rahimi B, et al. (2021) A combination of herbal compound (SPTC) along with exercise or metformin more efficiently alleviated diabetic complications through down-regulation of stress oxidative pathway upon activating Nrf2-Keap1 axis in AGE rich diet-induced type 2 diabetic mice. *Nutr. Metab.* 18(1):1-14
- Rasouli Vani J, Taghi Mohammadi M, Sarami Foroshani M, Rezazade E (2019)

- Evaluation of the neuroprotective and antioxidant effects of *Dorema aucheri* extract on cerebral ischaemia-reperfusion injury in rats. *Pharm Biol.* 57(1):255-262
- Safavi K, Abedpoor N, Hajibabae F, Kaviani E (2025) Mitigating diabetic cardiomyopathy: the synergistic potential of sea Buckthorn and Metformin explored via bioinformatics and chemoinformatics. *Biology.* 14(4):361
- Schmidt-Ott UM, Ascheim DD (2006) Thyroid hormone and heart failure. *Curr. Heart Fail. Rep.* 3(3):114-119
- Singh S, Panda V, Sudhamani S, Dande P (2020) Protective effect of a polyherbal bioactive fraction in propylthiouracil-induced thyroid toxicity in rats by modulation of the hypothalamic–pituitary–thyroid and hypothalamic–pituitary–adrenal axes. *Toxicol. Rep.* 7:730-742
- Szklarczyk D, Gable AL, Nastou KC, et al. (2021) The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* 49(D1):D605-D612
- Tang Y-D, Kuzman JA, Said S, Anderson BE, Wang X, Gerdes AM (2005) Low thyroid function leads to cardiac atrophy with chamber dilatation, impaired myocardial blood flow, loss of arterioles, and severe systolic dysfunction. *Circulation.* 112(20):3122-3130
- Wang H, Lian X, Gao W, et al. (2022) Long noncoding RNA H19 suppresses cardiac hypertrophy through the MicroRNA-145-3p/SMAD4 axis. *Bioengineered.* 13(2):3826-3839
- Wang L, Quan N, Sun W, et al. (2018) Cardiomyocyte-specific deletion of Sirt1 gene sensitizes myocardium to ischaemia and reperfusion injury. *Cardiovasc. Res.* 114(6):805-821