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Cardiac Hypertrophy Caused by Hyperthyroidism in Rats: Role of ATF-6 and TRPC1 Channels

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1 **Cardiac Hypertrophy Caused by Hyperthyroidism in Rats: Role of ATF-6 and**
2 **TRPC1 Channels**

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Cardiac Hypertrophy Caused by Hyperthyroidism in Rats: Role of ATF-6 and TRPC1 Channels

Abstract

Hyperthyroidism influences the development of cardiac hypertrophy. Transient receptor potential canonical channels (TRPCs) and ER stress are regarded as critical pathways in cardiac hypertrophy. Hence, we aimed to identify the TRPCs associated with ER stress in hyperthyroidism-induced cardiac hypertrophy.

20 adult Wistar albino male rats were used in the study. The control group was fed with standard food and tap water. The group with hyperthyroidism was also fed with standard rat food, along with tap water that contained 12 mg/L of thyroxine for four weeks. At the end of the fourth week, the serum-free T₃, T₄, and TSH levels of the groups were measured. The left ventricle of each rat was used for histochemistry, immunohistochemistry, western blot, total antioxidant capacity (TAC), and total oxidant status (TOS) analysis.

As per our results, ATF-6, IRE-1, and TRPC1, which play a significant role in cardiac hypertrophy caused by hyperthyroidism, showed increased activation. Moreover, TOS and ft₃ levels increased, while TAC and TSH levels decreased.

With the help of the literature review in our study, we could, for the first time, indicate that the increased activation, in particular of ATF-6, IRE-1, and TRPC1-induced deterioration of the Ca²⁺ ion balance, leads to hypertrophy in hyperthyroidism due to heart failure.

Keywords: Cardiac hypertrophy; hyperthyroidism; ATF-6; TRPC1

1 **Introduction**

2 The thyroid gland typically produces two hormones—thyroxine (T4) and
3 triiodothyronine (T3). Notably, thyroxine, which is the main form of a hormone produced
4 by the thyroid gland, often serves as a prohormone. Thyroid hormones typically affect all
5 organs of the human body, and the heart is the most affected; even the slightest change in
6 the serum concentration has a major effect on the heart. Hyperthyroidism is an illness in
7 which the thyroid gland produces more thyroid hormone than the body needs.
8 Hyperthyroidism usually increases heart rate, myocardial contractility, cardiac output,
9 and the left ventricular mass, along with supraventricular and ventricular arrhythmia
10 (Dillmann, 2010).

11 Thyroid hormone-responsive genes in heart muscle include thyroid hormone-induced α -
12 myosin heavy chain (MHC- α), sarcoplasmic reticulum's calcium pump (SERCa2), and
13 suppressed MHC- β . Increased thyroid hormone action significantly induces cardiac
14 protein synthesis and causes concentric cardiac hypertrophy. Thyroid hormone-induced
15 cardiac hypertrophy presents a physiological hypertrophy in the initial stages with
16 increased levels of the SERCa2 and decreased MHC- β expression (Kinugawa et al.,
17 2001). Prolonged exposure to high T3 levels may increase the synthesis of cardiac
18 proteins, leading to concentric cardiac hypertrophy and dysfunction (Osuna et al., 2017,
19 Dillmann, 2010).

20 The endoplasmic reticulum (ER) is an organelle that is involved in multiple cellular
21 processes, such as Ca^{2+} homeostasis, which is the folding of membrane proteins and lipid
22 biosynthesis (Ron and Walter, 2007). Different types of physiological damages in an
23 organism can affect its ER functionality, potentially resulting in the accumulation of

1 unfolded or improperly folded proteins in the ER. Recent studies have shown that
2 apoptosis induced by unfolded protein response (UPR) and ER contributes to the
3 pathophysiology of cardiovascular, neurodegenerative, diabetes mellitus, and liver
4 diseases (Lukas et al., 2019, Zhang et al., 2019). When ER stress occurs, three ER
5 transmembrane sensors—protein kinase-like ER kinase (PERK), inositol-requiring
6 kinase 1 (IRE-1), and activating transcription factor 6 (ATF-6)—are activated (Kim et
7 al., 2008). All of the three sensors are kept in an inactive state through an interaction with
8 the glucose-regulated protein 78 kDa (GRP78) at the N-terminus. When the unfolded
9 proteins accumulate in the ER, GRP78 releases them in order to allow the oligomerization
10 of these sensors, thereby initiating UPR.

11 In cardiac myocytes, thyroid hormone affects the expression of the gene encoding the
12 calcium pump of the sarcoplasmic reticulum and by this way it regulates the flow of Ca^{2+}
13 (Dillmann, 2010). The disruption of Ca^{2+} homeostasis in the ER is considered an
14 important trigger of ER stress. It has been shown that members of the transient receptor
15 potential (TRP) family mediate cellular Ca^{2+} homeostasis and induce ER stress through
16 different mechanisms. For example, TRPC1 (Selvaraj et al., 2012), TRPV1 (Gallego-
17 Sandín et al., 2009), and TRPC6 (Albarran et al., 2014) channels are expressed in both
18 the ER and plasma membrane. These channels are associated with Ca^{2+} homeostasis in
19 the ER. Additionally, TRPC3 and TRPC6 channels have been found to play a major role
20 in ER stress-induced apoptosis (Ampem et al., 2016). The researchers of some studies
21 have identified different types of TRP channel genes in the heart of different species. The
22 identified genes include the following: TRPC5 (Bush et al., 2006), TRPC6 (Kuwahara et
23 al., 2006), TRPM4 (Guinamard et al., 2004), TRPP1, and TRPP2 (Chauvet et al., 2002)
24 in the human heart tissue and/or myocytes; TRPC3/6, TRPV2/4, TRPM3/7, and TRPP2

in the rat heart tissue and/or myocytes; and TRPCM and TRPM4 in the rat cardiac myocytes (Inoue et al., 2009). In previous studies, it has been seen that TRPC3 channels regulate rat cardiac fibroblast proliferation by controlling Ca^{2+} entry (Zhang et al., 2012). In light of all this information, it can be indicated that TRP channels play a significant role in the mechanism of ER Ca^{2+} homeostasis, and the disruption of the functions of this channel leads to ER stress (Sukumaran et al., 2016).

In this study, we aimed to identify the sensors that play a significant role in inducing ER stress in cardiac hypertrophy caused by hyperthyroidism and the TRP channel involved in the process.

Material and Methods

Our study was approved by the Eskişehir Osmangazi University Local Animal Ethics Committee (decision no. 634 in meeting no. 117, 30.11.2017) and followed the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize animal suffering and reduce the number of animals. A total of 20 adult Wistar albino male rats (7–8 weeks old, 200-250 gr) from the Medical and Surgical Experimental Research Center (TICAM) were used in the study. During the period of the experimentation, the rats were kept in a controlled environment for 12 hours in light/dark illumination, a temperature of $24 \pm 2^\circ\text{C}$, and humidity of $55 \pm 5\%$. They were placed in standard plastic cages (three to five animals per cage) two weeks before the experiment, and ambient conditions were ensured for adaptation. The rats were randomly assigned to control and

hyperthyroidism groups. The control group (euthyroid) (n=10) was fed with standard rat food and tap water, while the hyperthyroidism group (n=10) was fed with standard rat food and tap water containing 12 mg/L thyroxine for four weeks (Araujo et al., 2006).

At the end of the fourth week, the rats were euthanized through the administration of an intramuscular injection of ketamine (45 mg/kg) and xylazine (5 mg/kg). At the end of the experiment, the blood samples of the rats were collected by making careful intracardiac puncture and were, subsequently, centrifuged at 11000 rpm for ten minutes. The serums were stored in tubes at a temperature of -80°C until the day of analysis for measuring the serum-free T3, T4, and TSH levels. The left ventricle part of the heart tissue was taken out, and each removed tissue was divided into two parts—one part was placed in 10% neutral formaldehyde for histochemistry and immunohistochemistry analyses, while the other remaining part was stored at -80°C for western blot, total antioxidant capacity (TAC), and total oxidant status (TOS) analyses.

Biochemical Analysis

The free T3 (YLA0127RA, Shanghai YL Biotech, China), free T4 (YLA0239RA, Shanghai YL Biotech, China), and TSH (YLA0047RA, Shanghai YL Biotech, China) levels were determined using the enzyme-linked immunosorbent assay method (ELISA). The TAC (YLA0311RA, Shanghai YL Biotech, China) and TOS (YLA1392RA, Shanghai YL Biotech, China) levels were measured using the spectrophotometric method explained in the procedure section of the kits. We prepared all the reagents, samples, and standards. Subsequently, we added the prepared samples, standards, and ELISA solutions, allowing them to react for about an hour at 37°C . Afterward, we washed the plate five times, added the Chromogen solutions A and B, and incubated the solutions for

ten minutes at 37°C for color development. Next, for stopping the reaction, we added the stop solution. The expression levels provided the OD value within ten minutes by using the BioTek microplate reader 800 in 450 nm, which was used to calculate the results.

Preparation and Investigation of Histological Preparations

In order to conduct an examination at the microscopic level, the heart tissue samples were kept in 10% neutral formaldehyde for 72 hours. Routine histological procedures were performed on these tissues so as to form paraffin blocks. Then, 5 µm serial sections were taken from the prepared paraffin blocks and added to the positive-charged slides. Hematoxylin-eosin stained sections were determined using a BX51 light microscope (Olympus Corporation, Tokyo, Japan), which was attached to a DP70 digital camera (Olympus Corporation, Tokyo, Japan). The camera had a lens magnification of 10×, an image bit depth of 24, space resolution data of 4080 × 3072 pixels, a horizontal and vertical resolution of 72 dpi, and an exposure time of 1/800 seconds.

Immunohistochemistry

For performing the immunohistochemistry examination, 5 µm sections were cut from each paraffin block, which was then put on the positive-charged slides and kept in an incubator at a temperature of 65°C for an hour. After they were passed through xylol and a series of decreasing-concentration alcohol, the sections were incubated with 3% hydrogen peroxide to prevent endogenous peroxidase activity. After antigen retrieval with a citrate buffer, the area of the tissue sections was drawn out with a pap-pen. The sections were washed three times for five minutes with PBS / 0.2% Triton-X-100, followed by an hour of goat serum blocking at room temperature. They were subsequently incubated with rabbit polyclonal ATF-6 (ab203119), rabbit polyclonal IRE-1 (YID5384),

1 mouse monoclonal PERK (sc377400), mouse monoclonal TRPC1 (sc133076), and rabbit
2 Polyclonal TRPC5 (GTX54861) overnight at 4°C in a humidity chamber. After washing
3 with PBS, the cells were incubated with a biotinylated goat anti-polyvalent secondary
4 antibody (TP-125-BN, Thermo Fisher Scientific, USA) for 10 min at room temperature.
5 After washing with PBS, the cells were treated for 10 min with streptavidin. After
6 washing again with PBS, the cells were kept in the AEC chromogen for 5 min. Then the
7 reaction was stopped by the tap water, and the ground was stained with hematoxylin.
8 Immediately after the wash, for conducting the immunohistochemical examination of the
9 preparations, the Olympus BX51 computer-aided imaging system was used. For
10 performing our immunohistochemistry analysis, we used the Image J 1.52p program. This
11 analysis was performed on 30 sections for each group. The percentage of the area stained
12 by subtracting the space region from the total area measurement of each image was found
13 using the following formula:

$$\text{Percentage of the stained area} = \text{Stained area} \times 100 / \text{Total area of the space}$$

15 ***Western Blot Analyses***

16 In order to determine the changes in the amount of specific protein in the left ventricle
17 heart tissues of the rats in both the hyperthyroidism and control (euthyroid) groups, the
18 heart tissues were cut into small pieces and the RIPA Lysis Buffer solution system that
19 contains lysis buffer, PMSF, protease inhibitor cocktail and sodium orthovanadate (sc-
20 24948) was added to 2 ml beaded tubes. Subsequently, the homogenization process was
21 performed in the bead homogenizer. Following this, the tubes were incubated at 4°C for
22 at least half an hour, and then the tissue lysates were centrifuged at 4°C for 20 minutes at
23 15,000 g. The supernatant containing the proteins was removed using a pipette and placed

in a new tube. Using the Qubit 2.0 device, protein determination was performed on the samples present in the supernatant. Afterward, 50 µg of the protein was electrophoresed with an SDS-PAGE Bio-Rad Mini Trans-Blot cell device and then transferred onto one PVDF membrane using the Bio-Rad Trans-Blot Turbo device. The membranes were blocked using a blocking buffer of 5% bovine serum albumin (BSA) for an hour at room temperature, following which we added mouse monoclonal GRP78 (sc166490), rabbit polyclonal ATF-6 (ab203119), rabbit polyclonal IRE-1 (YID5384), mouse monoclonal PERK (sc377400), mouse monoclonal TRPC1 (sc133076), goat polyclonal TRPC4 (ab111841), and mouse monoclonal β-actin (housekeeping protein) (sc47778) to each of the membrane and incubated them overnight at 4°C. The membranes were washed three times with a wash solution (TBST) for ten minutes and then incubated again with appropriate secondary antibodies (sc2005, sc2030). The membranes were washed three times with TBST for ten minutes. The expression levels of the proteins in the immunoreactive bands were monitored by the imaging system (C-Digit, Licor, Cambridge, United Kingdom), and the results were analyzed using Image J 1.49v.

Statistical Analyses

Statistical analyses were performed using an IBM SPSS 22.0 Statistics package program. The accepted significance level was $p < 0.05$. The Shapiro-Wilk test was used to determine the normal data distribution. Independent samples *t*-test was performed for the normally distributed data. At the same time, the Shapiro-Wilk test was also used to investigate the biochemical results' normal distribution. All experimental data were determined to be normal, and parametric tests were performed. The homogeneity of the variances was measured using Levene's test. Accordingly, an independent samples *t*-test was performed in cases where the variances were homogeneous.

1 **Results**

2 ***Biochemical Findings***

3 We observed an increased level of free T3 and free T4 ($p < 0.05$) in the samples collected
4 from the hyperthyroid group compared to those from the control group. While monitoring
5 the TSH level, we found a significant decrease in the level for the hyperthyroid group (p
6 < 0.05 , Figure 1), as compared to the control group. In the hyperthyroid group ($p < 0.05$),
7 it was seen that the TAC level was decreased, whereas the TOS was increased ($p < 0.05$,
8 Figure 2).

9 ***Hyperthyroidism Causes Cardiac Hypertrophy***

10 We characterized the different histopathological features of the control and
11 hyperthyroidism-induced cardiac hypertrophy groups and also analyzed the euchromatic
12 nuclei as well as the parallel placement; transverse stripes of the cardiac muscle fibers
13 were observed for the rats in the control group (Figure 3a). Hypertrophic myocardial cells
14 with large, dark, and heterochromatic nuclei were detected in the hyperthyroid group. The
15 vacuole and pyknotic nuclei were also found in the cytoplasm of the myocardial cells. In
16 addition, mast cells were observed more frequently (Figure 3b1-b3).

Increased Expression of ATF-6 in ER-Stress Pathways Causes Cardiac Hypertrophy

The GRP78 protein expression levels, the master regulator of the UPR, were found to be significantly enhanced in the heart tissues of the rats in the hyperthyroidism group when compared to the control group, accompanied by a remarkable increase in ATF-6. When a change in intergroup protein expressions was seen using the western blot method, GRP78 2.66- and ATF-6 3.3-fold increases were also observed in the hypertrophic heart. Besides the increment of ATF-6, IRE-1 and PERK were also increased, although increased expression of ATF-6 was dominant in our study. When a change in intergroup protein expressions was seen with the western blot method, IRE-1 1.39- and PERK 1.85-fold increases were also observed in the hypertrophic heart (Figure 4). When the change in the intergroup immunostaining expressions by immunohistochemistry method was seen, ATF-6 69-fold (0.69 / 0.01), IRE-1 65-fold (0.65 / 0.01), and PERK 48-fold (0.48 / 0.01) increases were observed in the hypertrophic heart (Figure 5). In this case as well, an increased expression level of ATF-6 was dominant.

TRPC1 Increase Dominates among TRP Channels in Cardiac Hypertrophy

TRPC1 protein expression levels were found to be significantly enhanced in the heart tissues of the rats in the hyperthyroidism group as compared to the control group, accompanied by a remarkable increase in ATF-6, which was determined using western blot analysis (Figure 4). Besides TRPC1, TRPC4 also showed a slight increment. When the change in the intergroup protein expressions was seen using the western blot method, the TRPC1 channel was found to have increased 2.02 times, while the TRPC4 channel increased 1.15 times (Figure 4). In the immunohistochemistry method, we compared the change observed in the intergroup immunostaining expressions of TRPC1 and TRPC5.

TRPC5 showed a 1.25-fold (0.64 / 0.51) increment, while TRPC1 showed a 1.79-fold (0.93 / 0.52) increase. Here as well, TRPC1 expression levels were determined to be significantly enhanced in heart tissues after the incidence of hyperthyroidism (Figure 5).

Discussion

In our study, we investigated the relationship between TRP channels and ER stress sensors involved in the mechanism of hypertrophy on the basis of previous studies that suggest that hyperthyroidism causes hypertrophy (Teixeira et al., 2018, Wang et al., 2018). Using this information, the interaction between the various TRP channels and ATF-6, PERK, and IRE-1 ER stress sensors was explained using several molecular methods. Biochemical, histopathological, immunohistochemical and western blot results showed that thyroxine induced cardiac hypertrophy caused to increase dominantly ATF6 and TRPC1 expression.

Hypertrophy is one of the common clinical manifestations of hyperthyroidism-induced cardiac tissue. The experimental hyperthyroidism models are key to the development of new therapies for the treatment of hypertrophy and heart failure.

In all mammalian cells, protein misfolding is controlled by the following ER transmembrane sensors: ATF-6, PERK, IRE-1 (Blackwood et al., 2019). When unfolded proteins accumulate in the ER, GRP78 releases these sensors and initiates UPR (Bertolotti et al., 2000). Consistent with the findings of many other researchers, we also show that the significant increase in GRP78 expression and UPR activation in patients who have experienced heart failure is related to the pathophysiology of heart failure (Dally et al., 2009). Left ventricular dilation, systolic dysfunction, and cardiomyocyte apoptosis have been observed in association with an increased expression of CHOP (C/EBP homologous

protein) that is involved in GRP78 and ER stress-induced apoptosis pathway in immunized rabbits against β -adrenoceptors (Mao et al., 2007). These findings indicate that ER stress plays a critical role in dilated and autoimmune cardiomyopathy. In the cultured rats' ventricular myocytes, the stimulation of β -adrenoceptors activated ER stress and induced apoptosis (Dalal et al., 2012). It is supposed that β -adrenoceptor antagonists may reduce cardiac hypertrophy and the severity of heart failure by inhibiting ER stress (Wang et al., 2018). Blackwood et al. stated that mTORC protein-induced expression of ATF-6 is required, as shown in our study, in order to develop cardiac hypertrophy due to an increase in heart pressure and exercise (Blackwood et al., 2019).

IRE-1 triggers the activation of XBP1. However, its role in cardiac hypertrophy is not yet fully understood. Chen et al. showed that the increased regulation of NOX4 causes cardiomyocyte hypertrophy induced by the hypertrophic stimuli of Xbp1 / NF- κ B signals (Chen et al., 2017). In our study, IRE-1 was less involved than ATF-6 in causing cardiac hypertrophy.

According to Liu et al., PERK was essential during the experiment for avoiding congestive heart failure in the cultured mice (Liu et al., 2014). In parallel with this result, there was a remarkable change in the expression of the PERK pathway, but the overexpression of ATF-6, as compared to IRE-1 and PERK, suggested that the ER stress developed from the ATF-6 pathway in hyperthyroidism-induced cardiac hypertrophy.

Heart failure is associated with abnormal usage of Ca^{2+} in the heart tissue (Minamino and Kitakaze, 2010). In addition, the deterioration of Ca^{2+} homeostasis in the ER is considered an important trigger for ER stress. The members of the TRP family have been seen to mediate cellular Ca^{2+} homeostasis and induce ER stress through different mechanisms.

1 Among the TRP sub-family, TRPCs are typical receptor-activated Ca^{2+} permeable cation
2 channels. A total of seven mammalian homologs (TRPC1-C7) have been identified to this
3 date and have been seen to be expressed in the heart. The TRPC family has an effect on
4 the cardiovascular system, especially in the cases of cardiovascular diseases. Since heart
5 failure develops with hypertrophy (Levy et al., 1990), hypertrophy mechanisms (Frey and
6 Olson, 2003) have been investigated in many studies. In parallel with the results of our
7 study, TRPC1, TRPC3, and TRPC6 were expressed in many murine hypertrophy models,
8 and their inhibition was used in the treatment of associated cardiovascular diseases (Eder
9 and Molkentin, 2011). An increased expression of TRPC5 and TRPC6 was observed in
10 the case of human heart failure (Bush et al., 2006, Kuwahara et al., 2006). Although the
11 subtypes of increased TRPC channels in hypertrophy differ between mice and humans,
12 these findings suggest that the expression of TRPC channels are increased in hypertrophy
13 for any anatomy, and it showed that the regulated TRPC may activate the local Ca^{2+}
14 signals required for pathological cardiac tissue remodeling and the progression of heart
15 failure. Kuwahara and Nishida have shown that TRPC3 and TRPC6 activated by
16 diacylglycerol (DAG) play important roles in the pathogenesis of cardiac hypertrophy
17 (Kuwahara et al., 2006). Mori also developed a pyrazole compound Pyr3 that selectively
18 inhibits TRPC3 and found that it suppresses cardiac hypertrophy in animal models, both
19 *in vitro* and *in vivo* (Nishida et al., 2015). The expression of cardiac TRPC3 is regulated
20 by a mechanism that is bound to calcineurin, which induces pathological cardiac
21 hypertrophy in rodent models. In contrast, TRPC5 is selectively induced in the case of
22 human heart failures. Functional studies have shown that TRPC channels can cause
23 cardiac hypertrophy through the activation of the calcineurin / NFAT signal. These results
24 indicate that TRPC channels play an important role in the transmission of pathological

Ca²⁺ signals to the heart (Bush et al., 2006). With an understanding of the role of TRPC1 and TRPC3 in hypertrophy, Wu et al. made use of TRPC4^{-/-} (negative dominant gene) mice. It was observed that the hypertrophy of TRPC4^{-/-} mice decreased in two weeks following the transverse aortic collapse caused by the disruption of Ca²⁺ flow (Wu et al., 2010). Two additional variants of TRPC4, TRPC4 α and TRPC4 β , have been identified (Freichel et al., 2014). In neonatal rat cardiomyocytes, the overexpression of TRPC4 α and non-expression of TRPC4 β increased the cell size and hypertrophy gene expression. In addition, a dominant negative TRPC4 mutant rat has been shown to reduce cellular hypertrophy induced by phosphoinositide phospholipase C β 1 (PLC β 1) (Cooley et al., 2014). Among all the TRPC channels we studied in our study, TRPC1 overexpression in particular in hyperthyroid-induced cardiac hypertrophy was thought to be responsible for the pathological change in the Ca²⁺ concentration in the ER, especially through the TRPC1 channel.

In conclusion, the results of our study firmly put ATF-6 in a critical position as a determinant of cardiac growth. Moreover, likely as a result of the deterioration of the TRPC1-induced Ca²⁺ ion balance, IRE-1 and especially ATF-6 is overexpressed, which leads to the development of hyperthyroidism-induced hypertrophy. Our findings underscore the widespread importance of the ATF-6/TRPC1 signaling axis described in the study of hyperthyroidism-induced cardiac hypertrophy. As a result of our literature review, we could indicate, for the first time, that hyperthyroidism causes disruption of TRPC1 and heart hypertrophy due to the overexpression of IRE-1 and especially ATF-6.

Limitations of the study

TRPC1 inhibitors could be also used to observe its clear role in cardiac hypertrophy in this experiment. Another limitation of this study is use of only male rats but not female rats.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

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Author contributions

N.E.B.A. and E.S. were involved in study conception and design. R.B., Ş.K., S.K., N.E.B.A. and E.S. were involved in data acquisition. V.S. and D.B.D. analyzed and interpreted data. N.E.B.A. and E.S. prepared the manuscript. All authors reviewed final form of the manuscript.

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Figure 1. Free triiodothyronine (fT3), thyroxine (fT4), and thyroid-stimulating hormone (TSH) levels, $*p < 0.05$.

Figure 2. Total antioxidant capacity (TAC) and total oxidant status (TOS) levels in tissue lysates, $*p < 0.05$.

Figure 3. Micrographs from hematoxylin- and eosin-stained rat heart myocardial tissues of control (a) and hyperthyroid (b1–3) groups. In the control group, healthy regulated myocardial fibers were observed. In the hyperthyroid group, vacuole (arrowhead), pycnotic nucleus (thin arrow), hypertrophic nucleus (thick arrow), and increased mast cells (stars) were observed. Scale bar = 50 μ m

Figure 4. GRP78, ATF-6, IRE-1, PERK, TRPC1, and TRPC4 protein levels were assessed by western blotting with the indicated antibodies in the control and hyperthyroid groups (n = 10 per group). The experiments were replicated three times. β -actin was used as an internal reference control. GRP78, ATF-6, IRE-1, PERK, TRPC1, and TRPC4 protein levels and graph of fold change. All proteins, especially ATF-6 and TRPC1, expressions increased, as revealed by the western blotting. The increase of all proteins was statistically significant (a, b, c, d, e, f. $p < 0.05$).

Figure 5. Micrographs from immunohistochemical-stained rat heart acquired with 40 $\times$ magnification, illustrating ATF-6, IRE-1, PERK, TRPC1, and TRPC5 with non or low (control group) (a) and high (hyperthyroidism group) (b) positive values for red/pink stain. The total positivity is presented in the boxes on the upper right side of each image. Scale bar = 50 μ m.

Figure 1

273x191mm (300 x 300 DPI)

Figure 2

166x59mm (300 x 300 DPI)

Figure 3
72x55mm (300 x 300 DPI)

Figure 4

258x150mm (300 x 300 DPI)

Figure 5

348x484mm (150 x 150 DPI)