MEDICINE UPDATES JOURNAL

Faculty of Medicine Port Said University

Volum: 23 No:3 PP:30 -43

" *Protective impact of Hesperidin in hyperthyroid-induced Cardiomyopathy in rats*"

Authors

Suzan A. Khodir[1*], Noha M. Abd El-aziz[2], Reem M. Emam[3], Heba Ashraf Elmalky[4], Mai M. Abdalraouf[5], Mohamed Awad Shahien[6], Reem Mohammed Ahmed[7], Tarek H. Abd-Elhamid [8,9], Rasha Arafa Abd Elmaksoud [10], Shorouk E. M. Elmorshdy[3]

[1] **Medical Physiology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.**
[2] **Anatomy and Embryology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.**
[3] **Medical Physiology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.**
[4] **Endocrinology unit, Internal Medicine Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.**
[5] Cardiology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt
[6] Clinical Pharmacology Department, Faculty of medicine, Damitta University, Egypt.
[7] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine (Girls), Al-Azhar University
[8] Histology and Cell Biology, Faculty of Medicine, Assiut University, Assiut, 71515, Egypt.
[9] Histology, Faculty of Medicine, Aqaba Medical Sciences University, Aqaba, 77110, Jordan
[10] Pathology Department, Faculty of Medicine, PortSaid University, Egypt.

Submitted: 07/03/2025

Accepted:15/03/2025

DOI: 10.21608/muj.2025.366265.1211

ISSN : 2682-2741

https://muj.journals.ekb.egdean@med.psu.edu.eg

vice_dean_postgraduate@med.psu.edu.eg

ABSTRACT:

Background: Higher risk of cardiovascular problems is linked to hyperthyroidism. The citrus bioflavonoid hesperidin (HSP) has cardioprotective, anti-inflammatory, and antioxidant impacts.

Aim of the study: to illustrate the possible underlying processes and cardioprotective impact of HSP in cardiomyopathy caused by hyperthyroidism.

Material and methods: Thirty male albino rats were split into three groups: Hyperthyroid, Hyperthyroid+HSP, and control (10/group). After four weeks rats were subjected to ECG evaluation, LVW/ tibial length, Serum TSH, Serum total T3, T4, Serum LDH, Serum CK-MB, Cardiac MDA, Cardiac SOD, Cardiac TNF-α, Cardiac IL-6, Cardiac genes expression of ANF, Caspase-3 and NF-kB evaluation. Furthermore, cardiac histological and immunohistochemical analyses were performed.

Results: There was dramatically elevated heart rate, LVW/ tibial length, serum tT3, serum tT4, serum LDH, serum CK-MB, cardiac MDA, cardiac TNF-α, cardiac IL-6, cardiac ANF gene expression, cardiac Caspase-3 gene expression and cardiac NF-kB gene expression alongside a marked reduction in duration of PR interval and QT interval, serum TSH and cardiac SOD of hyperthyroid group compared to control. HSP dramatically ameliorated hyperthyroid-induced cardiac alterations generated by hyperthyroidism.

Conclusion: In hyperthyroid-induced cardiomyopathy, HSP has a cardioprotective effect by enhancing ANF levels and regulating the heart's anti-inflammatory, anti-apoptotic, and antioxidant mechanisms.

KeyWords: ANF, Cardiomyopathy, Hesperidin, Hyperthyroid, NF-kB.

NTRODUCTION:

Thyroid hormones have a significant impact on the cardiovascular system in addition to basal metabolism. The regulation of oxygen consumption linked to an increase in thermogenesis is mediated by these hormones. Similarly, it is well established that thyroid hormones affect cardiac tissue, causing hypertrophy, increased heart rate, elevated cardiac output, and enhanced myocardial contractility **(Baraldi et al., 2013)**. Even slight variations in thyroid hormone levels can have a detrimental effect on the heart's electrophysiological characteristics and cardiac contractility **(Razvi et al., 2018).**

An increased risk of cardiovascular problems, such as arrhythmias, angina pectoris, cardiac hypertrophy, and heart failure, is linked to hyperthyroidism **(Jabbar et al., 2017)**. The term "hyperthyroidism-induced cardiomyopathy" describes a group of heart conditions brought on by hyperthyroidism. Heart failure, valvular disorders, cardiac hypertrophy, arrhythmia, and atrial fibrillation are some of its symptoms **(Klein & Ojamaa, 2001)**. It's unclear exactly how cardiomyopathy in hyperthyroidism works. Variable levels of oxidative stress in the body are associated with a hypermetabolic condition caused by hyperthyroidism; oxidative stress-related damage to proteins, lipids, and DNA has been documented in hyperthyroid rats (Ashry et al., 2023). Additionally, hyperthyroid people may be more susceptible to cardiovascular disease due to inflammation **(Shakoor et al., 2021)**.

Cardiomyocytes release atrial natriuretic peptide (ANP) in reaction to myocardial strain **(Garbers et al., 2006).** The homeostasis of cardiovascular volume and pressure is significantly regulated by ANP. In fact, ANP inhibits the renin-angiotensin-aldosterone system (RAAS) and causes diuresis, natriuresis, and vasodilation. ANP generation is elevated in pathologic circumstances like heart failure due to excessive RAAS activation, volume overload, and the resulting increased myocardial stretch. ANP acts as a compensatory reaction to the altered cardiovascular homeostasis by virtue of its unloading qualities **(Cannone et al., 2019)**. Patients with hyperthyroidism had higher ANF concentrations. These imply that thyroid hormone is one of the regulating variables for ANF and that its concentration is often elevated in hyperthyroidism **(Kohno et al., 1987)**.

Anti-hyperthyroid medications can cause adverse reactions, most notably severe liver damage, although many of them are potentially fatal. Managing these side effects is difficult and a major topic of discussion among endocrinologists at the moment. Due to their strong immunomodulatory, anti-carcinogenic, and antioxidant properties, plants high in natural phytochemicals have been the subject of extensive investigation in recent years **(Ashry et al., 2023).**

From an economic standpoint, citrus species are a valuable biological resource since they yield a variety of phytonutrients and phytochemicals with potential medical uses. The biological activity and bioavailability of flavonoids vary. Through their anti-inflammatory, antioxidant, antibacterial, antiproliferative, proapoptotic, and hormone-regulating qualities, these substances may offer health advantages **(Ali et al., 2023)**. A citrus bioflavonoid called hesperidin (HSP) is abundant in citrus fruits, as well as in a variety of vegetables, wine, and

green tea. Citrus trees yield a fruit called hesperidium, which is the source of HSP's name. Numerous pharmacological benefits of HSP have been demonstrated, including cardioprotective, anti-inflammatory, anticarcinogenic, and antioxidant properties **(Alharbi et al., 2023).**

Assessing the cardioprotective effect of HSP in rats subjected to hyperthyroid-induced cardiomyopathy and any potential underlying mechanisms implicated were the study's objectives.

Methods

Animals

Thirty mature male Wistar albino rats weighing 200 ± 50 g at three months of age were used in this study. They were kept under normal circumstances with a natural light-dark cycle in the animal house of the Faculty of Medicine at Menoufia University in Egypt. They were given free access to water and fed regular rat chow. Prior to the trial, rats were given a week to acclimate.

Ethical Statement

Every technique was carried out in accordance with the standards set forth by the Faculty of Medicine's Committee on Animal Research Ethics at Menoufia University with IRB NO: 3/2025ANAT17-1. We adhered to the ARRIVE reporting requirements.

Study design

Following a week of acclimatization to the lab environment, the rats were divided into three groups at random (10 rats each).

(1) Control group: Rats were not given any treatment and were given 1 milliliter of 0.9% normal saline solution i.p. every day for four weeks.

(2) Hyperthyroid group: Rats were administered 100 mg/kg of L-thyroxine sodium intraperitoneally (i.p.) daily for four weeks to induce hyperthyroidism **(Ibrahim et al., 2021).** A 100 mg Euthyrox tablet (Amoun Pharmaceutical Co. S.A.E. Egypt) was pulverized in 5 ml of saline to formulate L thyroxine, and the rat's weight was used to ascertain the volume of the intraperitoneal injection.

(3) Hyperthyroid/ Hesperidin treated group (Hyperthyroid + HSP) received Hesperidin (50 mg/kg/day, intraperitoneally) and L-thyroxine sodium (100 mg/kg, intraperitoneally) daily for four weeks. In accordance with previous protocols, HSP (CAS number: 520-26-3), sourced from Sigma-Aldrich (Germany), was solubilized in standard saline for a duration of four weeks **(Ashry et al., 2023)**.

Electrocardiograms (ECG) of all rats were recorded following a four-week period. On the second day, fasting blood samples were collected. The rats were subsequently euthanized via cervical decapitation while under light anesthesia following the determination of their final body weight. The hearts were subsequently excised, weighed, and readied for biochemical analysis, immunohistochemical assessment of caspase-3 and NF-kB, and histological examination utilizing hematoxylin and eosin (H&E).

ECG Method

The animals were placed on animal block boards for ECG recording following sedation with sodium pentobarbital (50 mg/kg i.p.). The Biopac ECG apparatus (Biopac Lab System, MP36R Unit and Acknowledge 5 Software, California, USA) was utilized to capture the ECG. The grounded electrode was affixed to the right hind leg, whereas disk electrodes were secured to the palmar surfaces of the forelimb and left hind limb. The heart rate, RR interval, and QT interval were monitored by Lead II, which was subsequently recorded and evaluated **(Kumar et al., 2017).**

To determine the extent of left ventricular hypertrophy, the ratio of left ventricle weight (LVW) to tibia length was calculated by sacrificing rats, followed by dissection and weighing of the left ventricle **(Husková et al., 2021).**

Blood collection

Blood samples were obtained from the retroorbital plexus of all rats. The blood samples were allowed to coagulate at room temperature for thirty minutes. Centrifugation was employed to isolate the serum for 15 minutes at 3000 revolutions per minute. Before the experiment, the serum was cryopreserved at -20 degrees Celsius.

Biochemical analyses

The levels of TSH, tT3, and tT4 were measured using rat ELISA kits that were acquired from Cusabio, China (catalogue numbers: CSB-E05085r; CSB-E05082r; and CSB-E05115r, respectively). The appropriate rat ELISA kits were used to evaluate the levels of LDH and CK-MB. Following the manufacturer's guidelines, use the Rat LDH ELISA Kit (Catalog No. MBS269777, MyBioSource Inc., San Diego, CA, USA) and Rat CK-MB ELISA Kit (Catalog No. MBS2515061, MyBioSource Inc., San Diego, CA, USA).

Tissue Homogenate Preparation

A tissue homogenizer (MPW120, MPW Medical Instruments, China) was employed to homogenize the individually weighed heart tissues. Following the centrifugation of the crude tissue homogenate, the supernatant was obtained and stored at -80°C for subsequent testing.

In accordance with the manufacturer's guidelines, an ELISA Kit was employed to evaluate cardiac TNF-α (Cat.: MBS2507393, MyBioSource, San Diego, CA, USA) and IL-6 (Cat.: MBS269892, MyBioSource, San Diego, CA, USA). Cardiac MDA and superoxide dismutase (SOD) were quantified in accordance with the manufacturer's guidelines utilizing calorimetric kits from Biodiagnostic Company, Dokki, Giza, Egypt **(Khodir et al., 2025 A).**

Real time PCR (rt-PCR) for detection of ANF, caspase-3 and NF-kB genes expression:

Cardiac ANF, caspase-3, and NF-kB were detected using Applied Biosystems' 7500 real-time PCR apparatus (CA, USA). The initial PCR phase was conducted following the extraction of RNA from cardiac tissue with the Direct-zol RNA Miniprep kit (Cat. No. R2051; Zymo Research, USA). Following the synthesis of complementary DNA with the QuantiTect Reverse Transcription Kit (205311, Qiagen, Applied Biosystems, USA), the subsequent rt-PCR step was performed utilizing the QuantiTect SYBR Green PCR Kit alongside pre-prepared Primer Assay (204143; Qiagen, USA) to quantify gene expression levels. The forward primer for ANF was (CCCAGCCTGAGAGTCCTACT), and the reverse primer was (TTTGAGCGCAATTGGAAGGC). The forward primer for caspase-3 was (GAGCTTGGAACGCGAAGAAA) and the reverse primer was (TAACCGGGTGCGGTAGAGTA). The NF-kB forward primer was (TCGACCTCCACCGGATCTTTC). The reverse primer was (GAGCAGTCATGTCCTTGGGT). β-actin forward, 5′-CCCATCTATGAGGGTTACGC-3′ and reverse, 5′-TTTAATGTCACGCACGATTTC -3′ as endogenous control **(Khodir et al., 2025 B).**

Histopathological Method

Cardiac tissue sections were fixed in 10% neutral buffered formalin (pH = 7.0) for histological analysis. They were subsequently desiccated in ethyl alcohol, rinsed with xylene, and ultimately stored in paraffin. Masson's Trichrome stain and Haematoxylin & Eosin were employed to stain the 4 μm-thick sections.

Heart paraffin sections (4 μm) were subjected to a 10-minute treatment with 3% hydrogen peroxide for immunohistochemical analysis. Primary antibodies targeting Caspase-3 (1:100 dilution, Elabscience Corp., Wuhan, China) and anti-NF-kB (monoclonal, 1:200 dilution, Abcam) were subsequently incubated for 30 minutes at 37 °C following blocking with BCA solution. Ultimately, slices were incubated for 30 minutes at ambient temperature with a secondary antibody conjugated to species peroxidase. Finally, the Image J program (Maryland, USA) was employed to evaluate the intensities of Caspase-3 and NF-kB immunostained fields in five randomly selected unique microscopic fields for each group **(Khodir et al., 2025 B).**

Statistical analysis

The statistical program version 16 of the Statistical program for the Social Sciences (SPSS) software was used to tabulate and analyze the data (SPSS, Inc., USA). Mean ± standard deviation (X±SD) was used to express quantitative data. One-way analysis of variance and a post hoc Tukey test were used to assess the significance of group differences. A p-value of less than 0.05 was deemed statistically significant.

Results

There was dramatically elevated heart rate, LVW/ tibial length, serum tT3, serum tT4, serum LDH, serum CK-MB, cardiac MDA, cardiac TNF-α, cardiac IL-6, cardiac ANF gene expression, cardiac Caspase-3 gene expression and cardiac NF-kB gene expression with substantial decline in duration of PR interval and QT interval, serum TSH and cardiac SOD of hyperthyroid group compared to control. Hyperthyroid+HSP group showed dramatically decreased heart rate, LVW/ tibial length, serum tT3, serum tT4, serum LDH, serum CK-MB, cardiac MDA, cardiac TNF-α, cardiac IL-6, cardiac ANF gene expression, cardiac Caspase-3 gene expression and cardiac NF-kB gene expression with

substantial elevation in duration of PR interval and QT interval, serum TSH and cardiac SOD compared to hyperthyroid group. Table (1), Fig (1)

Table (1): The measured Heart rate, PR interval, QT interval, LVW/ tibial length, Serum TSH, Serum tT3, Serum tT4, Serum LDH, Serum CK-MB, Cardiac MDA, Cardiac SOD, Cardiac TNF-α, Cardiac IL-6, Cardiac ANF gene expression, Cardiac Caspase-3 gene expression and Cardiac NF-kB gene expression in all studied groups

	Control group	**Hyperthyroid group**	**Hyperthyroid + HSP group**
Heart rate (beat/minute)	203±8.2	350±8.6 *	**270±6.9** *#
PR interval (millisecond)	30±1.15	17.6±1.2 *	**24.1±0.39** *#
QT interval (millisecond)	90±2.33	62±1.7 *	**76±1.02** *#
LVW/ tibial length (mg/mm)	13.6±1.06	18.6±0.78 *	**15.9±0.63** *#
Serum TSH (mIU/mL)	4.36±0.3	2.79±0.02 *	**3.39±0.14** *#
Serum tT3 (ng/dl)	70±2.3	140.5±3.12 *	**108.2±1.19** *#
Serum tT4 (ng/ml)	6.5±1.15	13.1±3.45 *	**9.23±0.8** *#
Serum LDH (U/L)	35.2±1.2	77.6±2.33 *	**58.±1.4** *#
Serum CK-MB (pg/mL)	43.6±3.9	141.5±4.23 *	**96.85±3.2** *#
Cardiac MDA (nmol/ gm. Tissue)	4.9 ±0.19	20.6± 1.9*	**13.2± 1. 4***#
Cardiac SOD (U/gm. Tissue)	7.36 ±0.13	5.11±0.13*	**6.23±0.06***#
Cardiac TNF-α (pg/ml)	121.6±7.1	250.99±5.19*	**187.6±4.1***#
Cardiac IL-6 (pg/mL)	190.6±6.36	380.3±3.4*	**287.9±6.87***#
Cardiac ANF gene expression	1	2.31±0.04*	**1.89±0.03***#
Cardiac Caspase-3 gene expression	1	3.25±0.08*	**2.23±0.09***#
Cardiac NF-kB gene expression	1	2.99±0.03*	**2.11±0.06***#

* Significant compared with control, # Significant compared with Hyperthyroid.

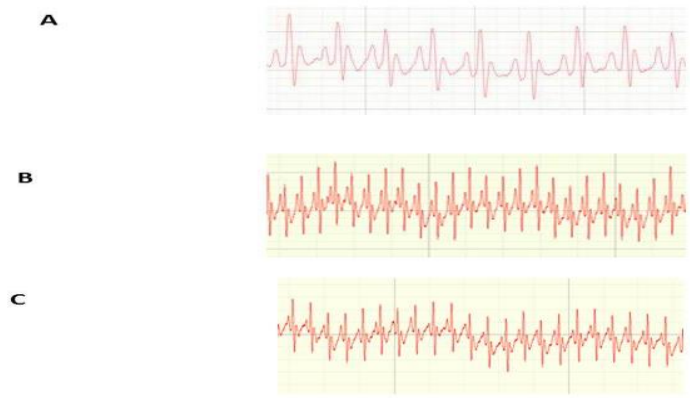

Fig. (1): ECG traces in all studied groups A) Control, B) hyperthyroid and C) Hyperthyroid+HSP groups.

Histolopathological results:

Control group's heart tissue stained with H&E revealed normal cardiac tissue architecture as branching and anastomosing cardiac muscle fibers with centrally placed oval nuclei and acidophilic sarcoplasm. The hyperthyroid group has a hemorrhagic region and cardiac muscle fiber damage and inflammatory cells infiltrations. The hyperthyroid+HSP group exhibits a tiny hemorrhagic region and improved cardiac tissue architecture but there is still little inflammatory cell infiltration × 400 magnification. (Fig 2: A-C)

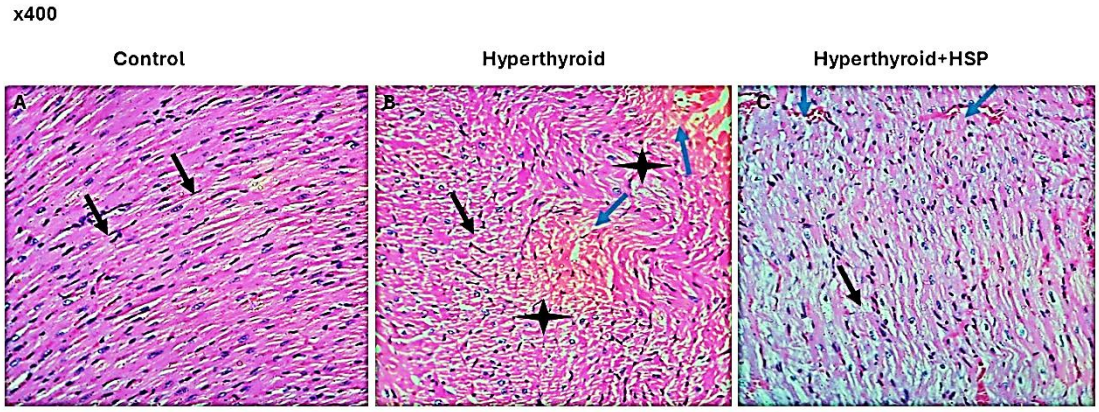

Fig. (2): Representative H&E histological sections of the cardiac tissue of **(A)** control group showed normal cardiac tissue with regular straiations (arrows)**. (B)** Hyperthyroid group showing irregular arrangement of muscle fibers (black arrow) with hemorrhagic areas (blue arrows) and inflammatory cells infiltrations (stars) **(C)** Hyperthyroid+HSP group showing amelioration in cardiac tissue architecture (black arrow) but there is still few inflammatory cell infiltration (blue arrows). . ×400 magnification.

The percentage area of Masson's Trichrome stained areas was dramatically higher in the hyperthyroid than control (48.6.6±0.22 vs. 7.8±0.15, respectively, $p<0.05$). This proportion, however, was significantly lower in the

Hyperthyroid+HSP group than in the Hyperthyroid group (16.2.8±0.45 vs. 48.6±0.22, respectively, p<0.05). (Fig. 3: A-D).

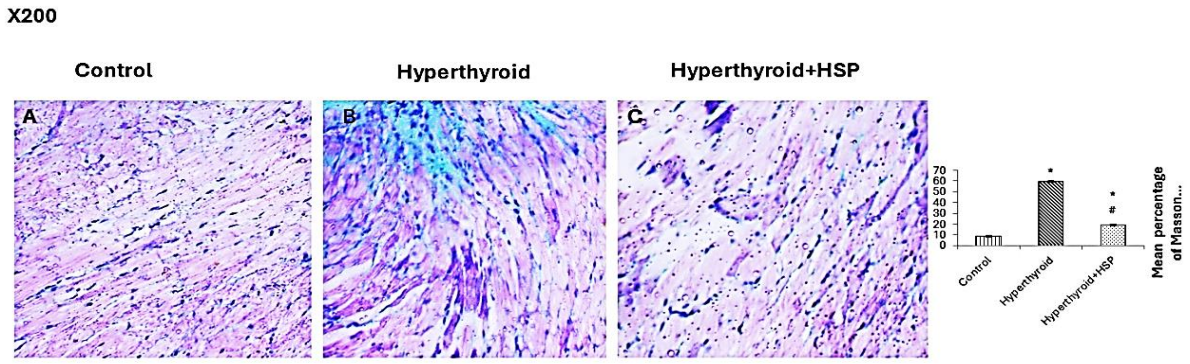

Fig (3): Micrographs of the different groups showing an elevation of the Masson Trichrome stain (A-D) in the Hyperthyroid group and a significant downregulation in the Hyperthyroid+HSP group.

Immunohistochemical results

When compared to the control, the hyperthyroid group's percentage area of caspase-3 increased significantly (80.4±0.41 vs. 10.5±0.33, respectively, p<0.05) in the Caspase-3 stain. However, compared to the Hyperthyroid group, this proportion significantly decreased in the Hyperthyroid+HSP group (30.2±0.05 vs. 80.4±0.41, respectively, p<0.05). (Fig. 4: A-D).

In the NF-kB stain, the percentage area of NF-kB was dramatically elevated in the hyperthyroid group than control group (78.5±0.23 vs. 9.8±0.45, p<0.05). This proportion, however, was significantly lowered in the Hyperthyroid+HSP group than in the Hyperthyroid group (29.4±0.35 vs. 78.5±0.23, p<0.05). (Fig. 4: E-H).

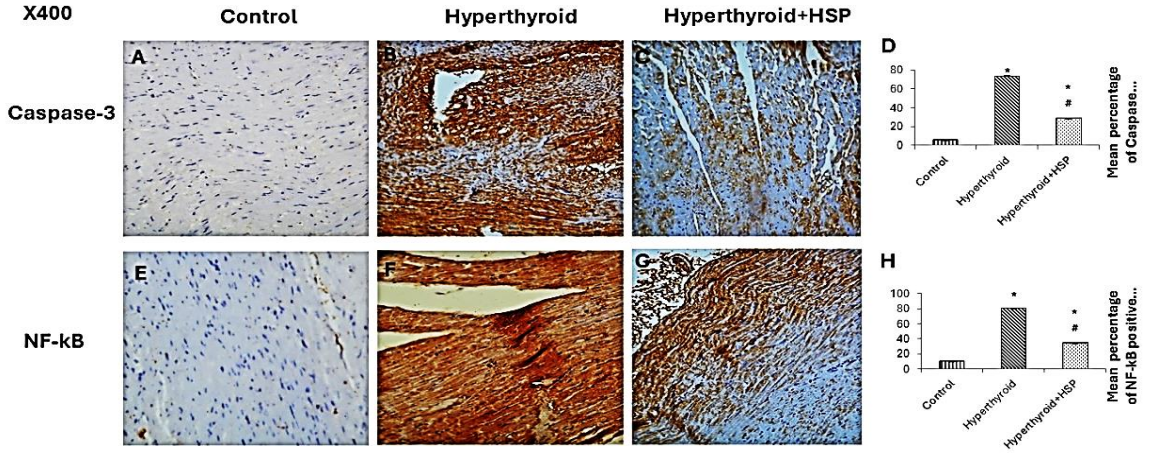

Fig (4): Micrographs of the different groups showing a substantial elevation of the Caspase-3 (A-D) and NF-kB (E-H) immunoreaction in the Hyperthyroid group and a significant decline in the Hyperthyroid+HSP.

Discussion

Hyperthyroid cardiac disease is a significant complication of hyperthyroidism. Heart failure is the initial clinical manifestation in approximately 6% of hyperthyroid individuals **(Siu et al., 2007).** Cardiomyopathy caused by hyperthyroidism may be reversible, contingent upon the timing and management of the hyperthyroid condition. Consequently, the prescription of cardioprotective medications may be advantageous for patients with hyperthyroidism. The cardioprotective benefits of HSP have been demonstrated in numerous studies **(Alharbi et al., 2023).** Consequently, the objective of this study was to investigate the potential cardioprotective effects of HSP in a rat model of hyperthyroid-induced cardiomyopathy.

The current investigation's demonstrated the effectiveness of inducing hyperthyroidism in rodents, as evidenced by the substantial decrease in TSH and the substantial increase in tT3 and tT4 in hyperthyroid rats compared to the control group. This is consistent with prior research **(Kim & Lee, 2019).**

The antihyperthyroidism potential of HSP was effectively demonstrated in this study by the successful reduction of T3 and T4 levels in hyperthyroidism-modeled animals. This is in accordance with a previous study **(Ashry et al., 2023)** and may be attributed to the antioxidant and free radical-scavenging properties of HSP, which have been confirmed by the significant improvement in oxidative status following hesperidin administration**.**

It has been established that THs induce cardiomyocyte hypertrophy in the heart. Hypertrophy may be a compensatory response to maintain cardiac output and enhance contractility. Nevertheless, this compensatory process can be decompensated by persistent stress, which can lead to extracellular remodeling, contractile failure, and reflected alterations in the gene expression profile **(Elnakish et al., 2015).**

As per the results of our investigation, hyperthyroid rats exhibited substantially elevated serum levels of cardiac biomarkers (LDH, CK-MB) in comparison to control rats. Furthermore, **Ibrahim et al. (2021)** observed a significant increase in LDH and CK-MP in a rat model of hyperthyroidism, suggesting a potential correlation between hyperthyroidism and cardiomyopathy. A significant increase in LVW/tibial length was associated with hyperthyroidism, as per **Zhang et al. (2013).** This impact was confirmed by histological analysis.

HSP is a citrus flavonoid. It possesses a variety of antioxidant and anti-inflammatory properties. HSP also offers protection against cardiac dysfunction by preventing myocyte apoptosis caused by pressure overload, inhibiting cardiac hypertrophy and fibrosis, and preventing oxidative stress **(Rezaee et al., 2021).** Compared to rats with hyperthyroidism, rats treated with HSP exhibited substantially lower cardiac biomarkers and LVW/tibial length. This is in accordance with our findings. The cardioprotective effects of HSP were also demonstrated in a previous study **(Alharbi et al., 2023).**

Additionally, the hyperthyroid group exhibited significant ECG alterations, such as an elevated heart rate, a reduced PR interval, and a decreased QT interval, in comparison to the control group, as demonstrated in a previous study (Zhang et al., 2013). These modifications may be attributed to hyperthyroidism, which is

characterized by increased L-type calcium channel expression and action potential shortening **(Bielecka-Dabrowa et al., 2009).**

The co-administration of HSP to hyperthyroid rodents resulted in a significant improvement in a variety of ECG parameters due to its capacity to stabilize membranes. HSP protects against lipid peroxidation by acting as an antioxidant for membranes. This was in accordance with our results, which demonstrated that the hyperthyroid HSP-treated group exhibited significantly lower levels of MDA, a lipid peroxidation product, than the hyperthyroid group.

The presence of oxidative stress in experimental hyperthyroidism was corroborated by the significant increases in MDA and decreases in SOD observed in the hyperthyroid group in comparison to the control group in this investigation. L-thyroxin-induced hyperthyroidism induces lipid peroxidation in a variety of rat tissues, including the heart **(Mogulkoc et al., 2006).** The hypermetabolic state that hyperthyroidism induces is the cause of systemic and cardiac tissue oxidative stress, which also generates free radicals **(Chainy & Sahoo, 2020). Mayyas et al. (2019)** discovered that cardiac SOD levels were substantially reduced in hyperthyroid rats.

The oxidative stress in animals with hyperthyroidism was significantly reduced by HSP. **Alharbi et al. (2023)** have previously reported on the antioxidant properties of HSP. **Mahmoud et al. (2017)** assert that hesperidin's antioxidative characteristics encompass the activation of Nrf2, the scavenging of free radicals, the inhibition of reactive oxygen species-generating enzymes, the reduction of DNA damage, and the enhancement of endogenous antioxidant defenses.

It is widely recognized that a key biochemical marker of cardiac hypertrophy is the synthesis of ANF. The rapid increase in cardiac ANF levels is associated with hyperthyroid-induced cardiac hypertrophy, similar to other hypertrophic stimuli **(De et al., 2004).** Our findings indicate that the cardiac ANF gene expression level in the hyperthyroid group was elevated compared to the control group, corroborating previous studies **(Chien et al., 1993).** The data indicated that serious cardiomyopathy is also induced by a prolonged hyperthyroid condition. In hyperthyroid rats treated with HSP, the ANF cardiac gene expression was considerably decreased compared to untreated hyperthyroid rats. The notable decrease in ventricular hypertrophy elucidates HSP's ability to diminish ANF, and its cardioprotective properties can be attributed to its anti-inflammatory and antioxidant characteristics.

A report indicates that hyperthyroidism increases NF-kB, a crucial factor for regulating gene transcription and the synthesis of inflammatory cytokines, which subsequently promote the generation of free radicals and lipid peroxidation **(Ashry et al., 2023).** This strategy aligns with the findings of the current study, which indicated that the hyperthyroid group had markedly elevated levels of pro-inflammatory cytokines and an increased cardiac NF-kB immunoreactivity and gene expression relative to the control group.

The inflammatory condition induced by hyperthyroidism was mitigated by HSP. **Mahmoud et al. (2017)** assert that HSP's ability to down-regulate NF-kB accounts for its anti-inflammatory properties. This aligns with our observations that HSP markedly diminished cardiac NF-kB gene expression and immunoreactivity. Moreover,

HSP-induced regulation of NF-kB and attenuation of inflammation may be facilitated by the activation of Nrf2 and HO-1. The Nrf2 protein is said to suppress the NF-kB pathway **(Cuadrado et al., 2014).** Our results align with a previous study **(Alharbi et al., 2023)** which demonstrated that HSP reduced the production of inflammatory mediators. Consequently, the current findings validated that HSP's robust anti-inflammatory characteristics were linked to its ability to address hyperthyroidism.

In cardiomyocytes, experimental hyperthyroidism induces DNA damage and increases caspase-3 activity **(Guler et al., 2022).** This was verified by the hyperthyroid rodents' heart's elevated expression of the apoptotic marker caspase-3 gene and immunoreaction **(Salem et al., 2023).**

HSP co-administration exhibited an antiapoptotic effect by downregulating caspase-3. HSP's antiapoptotic activity has previously been demonstrated in an experimental model **(Alharbi et al., 2023)**. HSP has been demonstrated to prevent apoptosis and cell cycle progression by connecting endoplasmic reticulum stress pathways, as per **Wang et al. (2015).**

The cardiac hyperthyroid group's upregulated masson trichrome stain was significantly reduced by HSP. The Masson Trichrome Stain has demonstrated that HSP significantly reduces inflammation and fibrosis due to its anti-inflammatory properties **(Alharbi et al., 2023)**.

Conclusion

In hyperthyroid-induced cardiomyopathy, HSP exerts a cardioprotective impact by decreasing ANF levels and modulating the heart's anti-inflammatory, anti-apoptotic, and antioxidant activities.

References :

Alharbi, F. K., Alshehri, Z. S., Alshehri, F. F., Alhajlah, S., Khalifa, H. A., Dahran, N., & Ghonimi, W. A. M. (2023). The role of hesperidin as a cardioprotective strategy against doxorubicin-induced cardiotoxicity: The antioxidant, anti-inflammatory, antiapoptotic, and cytoprotective potentials. *Open Veterinary Journal*, *13*(12), 1718.

Ali, Y. A., Ahmed, O. M., Soliman, H. A., Abdel-Gabbar, M., Al-Dossari, M., El-Gawaad, N. S. A., El-Nahass, E.-S., & Ahmed, N. A. (2023). Rutin and hesperidin alleviate paclitaxel-induced Nephrocardiotoxicity in Wistar rats via suppressing the oxidative stress and enhancing the antioxidant defense mechanisms. *Evidence-based Complementary and Alternative Medicine*, *2023*(1), 5068304.

Ashry, M., Askar, H., Obiedallah, M. M., Elankily, A. H., Galal El-Sahra, D., Zayed, G., Mustafa, M. A., El-Shamy, S. A. E.-M., Negm, S. A., & El-Beltagy, M. A. (2023). Hormonal and inflammatory modulatory effects of hesperidin in hyperthyroidism-modeled rats. *Frontiers in Immunology*, *14*, 1087397.

Baraldi, D., Casali, K., Fernandes, R. O., Campos, C., Sartório, C., Conzatti, A., Couto, G. K., Schenkel,

P. C., Belló-Klein, A., & Araujo, A. R. S. (2013). The role of AT1-receptor blockade on reactive oxygen species and cardiac autonomic drive in experimental hyperthyroidism. *Autonomic Neuroscience*, *177*(2), 163–169.

Bielecka-Dabrowa, A., Mikhailidis, D. P., Rysz, J., & Banach, M. (2009). The mechanisms of atrial fibrillation in hyperthyroidism. *Thyroid Research*, *2*, 1–7.

Cannone, V., Cabassi, A., Volpi, R., & Burnett Jr, J. C. (2019). Atrial natriuretic peptide: a molecular target of novel therapeutic approaches to cardio-metabolic disease. *International Journal of Molecular Sciences*, *20*(13), 3265.

Chainy, G. B. N., & Sahoo, D. K. (2020). Hormones and oxidative stress: an overview. *Free Radical Research*, *54*(1), 1–26.

Chien, K. R., Zhu, H., Knowlton, K. U., Miller-Hance, W., Van-Bilsen, M., O'Brien, T. X., & Evans, S. M. (1993). Transcriptional regulation during cardiac growth and development. *Annual Review of Physiology*, *55*(1), 77–95.

Cuadrado, A., Martín-Moldes, Z., Ye, J., & Lastres-Becker, I. (2014). Transcription factors NRF2 and NF-κB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation. *Journal of Biological Chemistry*, *289*(22), 15244–15258.

De, K., Ghosh, G., Datta, M., Konar, A., Bandyopadhyay, J., Bandyopadhyay, D., Bhattacharya, S., & Bandyopadhyay, A. (2004). Analysis of differentially expressed genes in hyperthyroid-induced hypertrophied heart by cDNA microarray. *Journal of Endocrinology*, *182*(2), 303–314.

Elnakish, M. T., Ahmed, A. A. E., Mohler, P. J., & Janssen, P. M. L. (2015). Role of oxidative stress in thyroid hormone-induced cardiomyocyte hypertrophy and associated cardiac dysfunction: an undisclosed story. *Oxidative Medicine and Cellular Longevity*, *2015*(1), 854265.

Garbers, D. L., Chrisman, T. D., Wiegn, P., Katafuchi, T., Albanesi, J. P., Bielinski, V., Barylko, B., Redfield, M. M., & Burnett, J. C. (2006). Membrane guanylyl cyclase receptors: an update. *Trends in Endocrinology & Metabolism*, *17*(6), 251–258.

Guler, G., Dasdelen, D., Baltaci, S. B., Sivrikaya, A., Baltaci, A. K., & Mogulkoc, R. (2022). The effects of thyroid dysfunction on DNA damage and apoptosis in liver and heart tissues of rats. *Hormone Molecular Biology and Clinical Investigation*, *43*(1), 47–53.

Husková, Z., Kikerlová, S., Sadowski, J., Alánová, P., Sedláková, L., Papoušek, F., & Neckář, J. (2021). Increased endogenous activity of the renin-angiotensin system reduces infarct size in the rats with early angiotensin II-dependent hypertension which survive the acute ischemia/reperfusion injury. *Frontiers in Pharmacology*, *12*, 679060.

Ibrahim, R. Y. M., Saber, A. A., & Hammad, H. B. I. (2021). The possible role of the seaweed Ulva fasciata on ameliorating hyperthyroidism-associated heart inflammations in a rat model. *Environmental Science and Pollution Research*, *28*, 6830–6842.

Jabbar, A., Pingitore, A., Pearce, S. H. S., Zaman, A., Iervasi, G., & Razvi, S. (2017). Thyroid hormones and cardiovascular disease. *Nature Reviews Cardiology*, *14*(1), 39–55.

Khodir, S. A., El-Haroun, H., Shukry, A. M., H Abd-Elhamid, T., Seddek, A. A., Samy, E., ... & Ahmed, K. E. S. (2025 A). The Rho Kinase Inhibitor Fasudil Mitigates Neurocognitive Deficits in D-Galactose-Induced Brain Aging in Rats: Involvement of SIRT1, BDNF and NF-κB. *Bulletin of Egyptian Society for Physiological Sciences*, *45*(1), 18-31

Khodir, S. A., Gaafar, A. M., Amer, M. S., Wahb, A., El-aziz, A., Elfakhrany, A., ... & Hussein, S. (2025 B). Renoprotective Effect of Maresin-1 in Deoxycorticosterone Acetate-Salt-Induced Hypertension in Rats: Targeting TLR4/NF-kB Pathway. *Bulletin of Egyptian Society for Physiological Sciences*, *45*(1), 123-137.

Kim, M., & Lee, B.-C. (2019). Therapeutic Effect of Scutellaria baicalensis on L-Thyroxine-Induced Hyperthyroidism Rats. *Evidence-Based Complementary and Alternative Medicine*, *2019*(1), 3239649.

Klein, I., & Ojamaa, K. (2001). Thyroid hormone and the cardiovascular system. *New England Journal of Medicine*, *344*(7), 501–509.

Kohno, M., Murakawa, K.-I., Yasunari, K., Nishizawa, Y., Morii, H., & Takeda, T. (1987). Circulating atrial natriuretic peptides in hyperthyroidism and hypothyroidism. *The American Journal of Medicine*, *83*(4), 648–652.

Kumar, P., Srivastava, P., Gupta, A., & Bajpai, M. (2017). Noninvasive recording of electrocardiogram in conscious rat: A new device. *Indian Journal of Pharmacology*, *49*(1), 116–118.

Mahmoud, A. M., Mohammed, H. M., Khadrawy, S. M., & Galaly, S. R. (2017). Hesperidin protects against chemically induced hepatocarcinogenesis via modulation of Nrf2/ARE/HO-1, PPARγ and TGF-β1/Smad3 signaling, and amelioration of oxidative stress and inflammation. *Chemico-Biological Interactions*, *277*, 146–158.

Mayyas, F., Alsaheb, A., & Alzoubi, K. H. (2019). The role of fish oil in attenuating cardiac oxidative stress, inflammation and fibrosis in rat model of thyrotoxicosis. *Heliyon*, *5*(12).

Mogulkoc, R., Baltaci, A. K., Oztekin, E., Sivrikaya, A., & Aydin, L. (2006). Effects of hyperthyroidism induced by L-thyroxin administration on lipid peroxidation in various rat tissues. *Acta Biologica Hungarica*, *57*(2), 157–163.

Razvi, S., Jabbar, A., Pingitore, A., Danzi, S., Biondi, B., Klein, I., Peeters, R., Zaman, A., & Iervasi, G. (2018). Thyroid hormones and cardiovascular function and diseases. *Journal of the American College of Cardiology*, *71*(16), 1781–1796.

Rezaee, R., Sheidary, A., Jangjoo, S., Ekhtiary, S., Bagheri, S., Kohkan, Z., Dadres, M., Oana Docea, A., Tsarouhas, K., & Sarigiannis, D. A. (2021). Cardioprotective effects of hesperidin on carbon monoxide poisoned in rats. *Drug and Chemical Toxicology*, *44*(6), 668–673.

Salem, H. R., Hegazy, G. A., Abdallah, R., & Abo-Elsoud, R. A. A. (2023). Protective role of vitamin D3 in a rat model of hyperthyroid-induced cardiomyopathy. *Journal of Traditional and Complementary Medicine*, *13*(3), 277–284.

Shakoor, S. A. K. K. A., Jiao, H. L., Tan, A. W. K., & Huiling, L. (2021). The role of inflammation in contributing to vascular risk in subclinical hyperthyroidism: randomized controlled trial. *Journal of Endocrinology and Metabolism*, *11*(1), 28–32.

Siu, C.-W., Yeung, C.-Y., Lau, C.-P., Kung, A. W. C., & Tse, H.-F. (2007). Incidence, clinical characteristics and outcome of congestive heart failure as the initial presentation in patients with primary hyperthyroidism. *Heart*, *93*(4), 483–487.

Wang, Y., Yu, H., Zhang, J., Gao, J., Ge, X., & Lou, G. (2015). Hesperidin inhibits HeLa cell proliferation through apoptosis mediated by endoplasmic reticulum stress pathways and cell cycle arrest. *BMC Cancer*, *15*, 1–11.

Zhang, Y., Dedkov, E. I., Teplitsky, D., Weltman, N. Y., Pol, C. J., Rajagopalan, V., Lee, B., & Gerdes, A. M. (2013). Both hypothyroidism and hyperthyroidism increase atrial fibrillation inducibility in rats. *Circulation: Arrhythmia and Electrophysiology*, *6*(5), 952–959.