Extra Virgin Olive Oil Protected Against Hyperthyroidism-Induced Cardiac Dysfunction Via Reduced Angiotensin-II Receptor Expression in Adult Male Rats

Original Article

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ABSTRACT

Background: Hyperthyroidism-induced cardiac dysfunction is a common disorder that was linked to the interplay between oxidative stress and activated renin-angiotensin system. Studies related the consumption of extra virgin olive oil (EVOO) to the lower risk of cardiovascular diseases.

Aim of the Work: So, this study aimed to evaluate the cardioprotective effects of extra virgin olive oil (EVOO) in experimentally induced hyperthyroidism with possible involvement of angiotensin-II receptor 1 (Ang-II-R1) and the antioxidant hemeoxygenase-1 (HO-1) enzyme.

Methods: Thirty adults male Wistar rats, allocated into 3 groups, control, Hyperthyroid group; received intraperitoneal injection of 100µg/kg L-thyroxine daily for 4 consecutive weeks, EVOO-treated hyperthyroid group; received L-thyroxine as in group II, then by the beginning of the third week, 1ml/100g EVOO by gavage daily for 2 weeks.

Results: EVOO-treated hyperthyroid rats showed significant body weight reduction with PR interval restoration near the control level. EVOO resulted in significant reduction in Ang-II-R mean % area and optical density with consequent reduction in the cardiac HO-1. This reduction was correlated with the improvement in basal cardiac inotropic function and cardiac inotropic response following isoproterenol infusion together with partial reversal of the hyperthyroid induced histological changes.

Conclusion: Prophylactic use of EVOO in hyperthyroidism could minimize the cardiac dysfunction and structural alterations by reducing Ang-II-R1 overexpression and mitigating oxidative stress.

Key Words: Ang-II-R1, cardiac dysfunction, extra virgin olive oil, hyperthyroidism, HO-1.

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INTRODUCTION

Hyperthyroidism is a widespread endocrine disorder that causes both functional and structural changes in the cardiovascular system[1]. ***Osuna et al.***[2] claimed that excess thyroid hormone can be associated with chronic hemodynamic overload, pathological cardiac remodelling, progressive contractile dysfunction, abnormal diastolic compliance predisposing to heart failure.

Jeppesen et al.[3] reported the presence of angiotensin receptors in cardiomyocytes and assumed their role in cardiovascular pathology. ***Diniz et al.***[4] stated that angiotensin receptor 1 is critical for the development of thyroid hormone-induced cardiac hypertrophy. ***Araujo et al.***[5] detected a close relationship between hyperthyroidism, oxidative stress and renin-angiotensin system (RAS) activation. Further, hyperthyroidism might increase cardiac renin activity and angiotensin II (Ang II) levels ***Barreto-Chaves et al.***[6].

Many epidemiological studies related the consumption of extra virgin olive oil (EVOO) to a lower risk of cardiovascular disease[7]. EVOO components as monosaturated oleic acid, polyunsaturated fatty acids,

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α-tocopherol (vitamin E) and vitamin K, as well as a wide variety of phenolic compounds such as tyrosol, hydroxytyrosol and oleuropein demonstrate potent antioxidant and anti-inflammatory functions[8].

Tanideh et al.[9] reported that EVOO lowered the levels of colonic malondialdehyde, myeloperoxidase activity, and interleukin-1β significantly in a rat model of acetic acid induced ulcerative colitis. Moreover, the use of EVOO in aged rats decreased aging-induced inflammation and oxidative stress in the liver, cardiac and aortic tissue[10]. Further, ***Alcaide-Hidalgo et al.***,[11] claimed angiotensin converting enzyme inhibitory activity of olive oil. Also, ***Domínguez-Vías et al.***,[12] suggested a beneficial effect of VOO in the regulation of rat systolic blood pressure through changes in renal aminopeptidases.

Hemeoxygenase-1 (HO-1) is an enzyme that demonstrate high antioxidant, anti-inflammatory, and antiapoptotic properties, it is normally expressed at low levels in most of organs, however, it is highly inducible in response to a variety of stimuli to protect cells against oxidative and inflammatory injury[13]. ***Hu et al.***[14] demonstrated that HO-1 attenuates Ang II–induced cardiac hypertrophy both in vitro and in vivo. Some studies stated that olive oil components as hydroxytyrosol may increase the expression of hemeoxygenase-1[15].

AIM OF THE STUDY

Therefore, the aim of the present study was to evaluate the possible cardioprotective effects of extra virgin olive oil (EVOO), as a natural antioxidant, in experimentally induced hyperthyroidism in rats. Also, to elucidate its effect on cardiac Angiotensin-II-Receptor 1 and cardiac HO-1 as a possible underlying mechanism.

MATERIALS AND METHODS

Experimental animals:

The study was performed on thirty adults male Wistar rats, initially weighing 200-300g. Rats were purchased from VACSERA HELWAN and were kept at the Faculty of Medicine Ain Shams Research Institute (MASRI), Ain Shams University, under standard conditions of boarding in animal cages (5 rat/cage) with suitable ventilation, temperature of 22-25°C and were subjected to normal light/dark cycle. Rats were fed regular meals in the form of bread and milk with free access to water and food ad libitum. The experimental procedures were started after one week of acclimatization.

ETHICAL DECLARATION

The study protocol was approved by the Research Ethical Committee of Faculty of Medicine Ain Shams University that follows the National Research Council of the National Academies guidelines (FWA000017585; FMASU MS 47/2019). Animals were handled with maximal care and hygiene without being subjected to any unessential stress or pain. Sample size was calculated using the G power program setting power at 80%, α error at 0.05 and 0.7 effect size using F test (ANOVA: fixed effects, omnibus, one way) and assuming a dropout of 10%, a sample size of at least 10 rats per group was recommended. The study was performed in accordance with ARRIVE guidelines.

Experimental groups:

Rats were randomly and blindly allocated into three equal groups (10 rats each);

Group I: Control group: Rats in this group received intraperitoneal (IP) injection of the solvent of L-thyroxine (a mixture of 96% alcohol and 1N NaOH isotonic saline), once daily, six days per week for 4 consecutive weeks.

Group II: Hyperthyroid group: rats in this group received i.p. injection of L-thyroxine, once daily, six days per week for 4 consecutive weeks in a dose of 100µg/kg/day[16].

Group III: EVOO-treated-Hyperthyroid group: rats in this group received IP of L-Thyroxine as in group II, by the beginning of the third week, EVOO was given once daily by gavage in a dose of 1ml/100g/day[17], for 2 consecutive weeks.

Drugs and chemicals:

- L-Thyroxine: L-Thyroxine was supplied by (sigma, USA) as a powder. Stock solutions were prepared by dissolving 4.5 mg L-thyroxine in 3ml solvent consisting of a mixture of 96% ethyl alcohol (2ml) and 1N NaOH isotonic saline (1ml) making final concentration of 1.5mg/ml, to be used in induction of hyperthyroidism.

- Extra virgin olive oil (EVOO): EVOO was obtained from Sinai, Egypt (fatty acids composition is 75% monosaturates, 13% polyunsaturates and 12% saturates, and phenolic concentration is 200 mg/kg).

Experimental procedures:

Rats were weighed and nasoanal length was estimated at the beginning and at the end of the study to calculate percent change in body weight and body mass index.

On the day of sacrifice at the end of the study (end of the 4th week), retroorbital blood samples were collected from overnight fasted rats, centrifuged at 4000 rpm for 15 minutes to obtain serum. Then, rats received IP heparin sodium 5000 IU/kg B.W. (Nile Company, Egypt). Fifteen minutes later, rats were anaesthetized with i.p. injection of thiopental sodium (EIPICO, Egypt), in a dose of 40 mg/kg B.W. When the stage of surgical anaesthesia had been reached as judged by loss of withdrawal reflexes ECG tracing was recorded using ECG recorder (Cardimax Fx-2111, Fukuda Denshi Co., Ltd., Japan).

The heart was dissected and immediately placed in ice-cold modified Krebs-Henseleit Bicarbonate (KHB) buffer solution to ensure rapid cardioplegia. In vitro study of isolated perfused heart was performed in Langendorff preparation according to the ordinary technique of Langendorff (1895) modified by Ayobe and Tarazi[18] using modified Krebs-Henseleit bicarbonate buffer (KHB) as perfusion medium.

Baseline developed peak tension (PT in g), time to peak tension (TPT in msec), rate of tension development (dT/dt in g/msec) and half relaxation time (HRT in msec) were recorded using (UGO BASILE S.R.L. Biological Research Apparatus 21036, Model 17304, Serial N. O448A15, Italy) connected to a computer provided with iWorx LabScribe2 Data Recording and Analysis Software program. Basal myocardial flow rate (MFR in ml/min) was determined by volumetric collection of the fluid passing out of the heart for 3 minutes. Peak developed tension and myocardial flow rate were calculated relative to the left ventricular weight (PT/LV, g/100 mg; MFR/LV, ml/100mg/min).

After recording basal cardiac activities, isoproterenol was infused through a catheter tube (PE-50, Clay Adams, New Jersey), connected to an opening just above the aortic cannula using a Sega-355 infusion pump in 5 consecutive rates of 0.324, 0.648, 0.864, 1.296, and 1.728 ml/minute for 3 minutes each, to obtain final doses of 0.65, 1.3, 1.73, 2.59, and 3.46 µg/minute. Then cardiac activities were recorded, and the myocardial flow was collected and measured. Three minutes after infusion of the last dose of isoproterenol, another record of the cardiac activities was obtained to show cardiac recovery. Maximal responses to isoproterenol and delta changes (the difference between the maximal responses and the basal values) were calculated. To prepare the isoproterenol infusion, it was weighted and dissolved in Krebs Henseleit bicarbonate buffer giving a final concentration of (2µg/ml).

Heart was then dried by filter paper and weighed in 5-Digit-Metler balance (AE 163). The left ventricle specimens were used for determination of cardiac tissue of hemeoxygenase 1 (HO-1) enzyme, other specimens were preserved in formalin for histopathological examination and immunohistochemical analysis of angiotensin II receptor 1. Body weight percent change (%BW) and body mass index percent change (%BMI) were calculated.

Biochemical Analysis:

- Serum free T3, T4, TSH were measured by an automated quantitative enzyme-linked fluorescent immunoassay (ELFA) technique using kits supplied by bioMérieux company, USA (CAT. No. 30 403-01, 30 404-01 and 30 400-01, respectively) with microplate reader set to 450 nm.

- Cardiac tissue HO-1 was measured by an enzyme linked immune-sorbent assay (ELISA) technique using rat specific kits supplied by abcam company, USA. (CAT. No. ab279414). Only those wells that contain HO-I, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color that was measured spectrophotometrically at a wavelength of 450 nm.

Histopathological Examination:

From all groups, specimens from the left ventricle of the heart (apex), were taken twice before and after the exposure to isolated heart studies, then they were immediately fixed in 10% formalin, to be processed and embedded in paraffin for histological analysis. Tissues were sectioned at 5µm and stained with hematoxylin and eosin (H&E).[19] Also, sections were used in immunohistochemical staining for angiotensin II receptor1[20].

- Immunohistochemical staining for angiotensin II receptor 1:

Sections were deparaffinized and rehydrated. Then, sections were microwaved for 15 minutes for antigen retrieval. After that, they were treated with a blocking solution for 20 min then incubated with 1:200 anti-Ang-

II-R1 polyclonal primary antibody (Sigma, SAB3500209) at room temperature for half an hour. Later, secondary antibody was added for 10 minutes. Finally, one to two drops of DAB (diaminobenzidine) were added for 10 minutes. At last, heart sections were counterstained with Mayer's hematoxylin, then dehydrated and cleared. Angiotensin II receptors expressed brownish coloration in cardiomyocytes.

- Histomorphometric analysis:

The mean area percentage and the mean optical density of angiotensin II receptor immunostaining were measured in 10 fields for each group. This was performed using the image analyzer Leica Q Win V.3 program installed on a computer in the Histology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM 2500 microscope with built in camera (Wetzlar, Germany).

Statistical analysis:

The normality of data was assessed by the Shapiro test. Parametric data were expressed as mean± SD (Standard deviation), statistical significance for data was determined using a one-way analysis of variance (ANOVA) with post-hoc Bonferroni test to find inter-group significance.

For non-parametric data median and interquartile range (IQR) were used, statistical significance was determined using Kruskal Wallis test followed by pairwise comparison and significance value was adjusted by the Bonferroni correction. Friedman's test was used for differences within the same group.

Spearman correlation was done to find the relation between parameters and the level of significance was accepted as $P < 0.05$. Statistical tests and significance were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc.) version 20.0.[21].

RESULTS

Anthropometric Measures:

The mean ranks of BW% were (24.56, 13.70 and 7.70) and of the BMI% were (21.89, 13.90 and 9.90) in control, hyperthyroid and EVOO-treated-hyperthyroid groups respectively. There was statistically significant difference in BW% ($X^2(2) = 18.92$, $p<0.001$) and BMI%($X^2(2) = 9.65$, $p<0.01$) between the three studied groups. After Bonferroni adjusted pairwise comparison, BW% showed significant differences between control group and each of hyperthyroid ($Z=10.86$, $p<0.02$) and EVOO-treated-hyperthyroid ($Z=16.86$, $p<0.001$) groups. Also, BMI% showed a significant difference between control and EVOO-treated-hyperthyroid ($Z=11.99$, $p<0.01$) group. (Table 1)

Cardiac weights

The mean ranks of RV/BW were (5.72, 17.50 and 20.85), of LV/BW were (5.00, 17.10 and 21.90) and of WH/BW were (5.00, 17.40 and 21.60) in control, hyperthyroid and EVOO-treated-hyperthyroid groups respectively. There was statistically significant difference in RV/BW ($X^2(2) = 16.28$, $p<0.001$), LV/BW ($X^2(2) = 19.59$, $p<0.001$) and WH/BW ($X^2(2)=19.26$, $p<0.001$) between the three studied groups. After Bonferroni adjusted pairwise comparison, significant differences exist between control and hyperthyroid groups ($Z= -11.78$, $p<0.01$) in RV/BW, ($Z= -12.10$, $p<0.01$) in LV/BW and ($Z= -12.40$, $p<0.01$) in WH/BW. Also, there were significant differences between control and EVOO-treated-hyperthyroid groups ($Z= -15.13$, $p<0.001$) in RV/BW, ($Z= -16.90$, $p<0.001$) in LV/BW and ($Z= -16.60$, $p<0.001$) in WH/BW. (Table 1)

Electrocardiographic (ECG) findings:

The mean ranks of HR were (5.56, 19.25 and 19.25), of PR were (19.50, 7.50 and 18.45), of QRS duration were (7.50, 13.25 and 23.50) and of R voltage were (7.22, 18.45 and 18.55) in control, hyperthyroid and EVOO-treated-hyperthyroid groups respectively. There was significant difference in HR ($X^2(2) = 16.92$, $p<0.001$), PR duration ($X^2(2)=18.28$, $p<0.001$), QRS duration ($X^2(2)=20.44$, $p<0.001$) and R voltage ($X^2(2)=11.13$, $p<0.01$) between the three studied groups.

After Bonferroni adjusted pairwise comparison, the significant differences in HR were only between control group and each of hyperthyroid and EVOO-treated-hyperthyroid ($Z= -13.69$, $p<0.002$) groups. The significant difference in PR duration was between control and hyperthyroid ($Z=12.00$, $p<0.01$) groups and between hyperthyroid and EVOO-treated-hyperthyroid ($Z= -10.95$, $p<0.002$) groups. Also, there were significant differences in QRS duration only between control and EVOO-treated-hyperthyroid ($Z= -16.00$, $p<0.001$) groups, and between hyperthyroid and EVOO-treated-hyperthyroid ($Z= -10.25$, $p<0.02$) groups. In addition, significant differences in R voltage were only between control group and each of hyperthyroid ($Z= -11.23$, $p<0.02$), EVOO-treated-hyperthyroid ($Z= -11.33$, $p<0.02$) groups. (Table 1).

Table 1: Median and interquartile range (IQR) of percentage change of BW (%BW), BMI (%BMI), relative weights of right ventricle (RV/BW), left ventricle (LV/BW), whole heart (WH/BW) and electrocardiographic findings; heart rate (HR), PR interval, QRS duration, R voltage, observed Q-T interval (Q-To), and corrected Q-T interval (Q-Tc interval) in all studied groups.

	Control	Hyperthyroid	EVOO-treated-Hyperthyroid
%BW	18.63	-0.94[a]	-9.64[a]
	(14.70-23.08)	(-8.12-3.32)	(-17.30- -5.09)
%BMI	7.98	-17.44	-18.72[a]
	(-7.87-16.74)	(-19.41-4.71)	(-20.04- -8.94)
RV/BW (mg/g)	0.92	1.35[a]	1.50[a]
	(0.69-1.10)	(1.27-1.49)	(1.30-1.88)
LV/BW (mg/g)	1.15	1.72[a]	1.87[a]
	(1.01-1.33)	(1.61-1.84)	(1.73-2.07)
WH/BW (mg/g)	2.30	3.31[a]	3.52[a]
	(1.85-2.49)	(3.15-3.46)	(3.18-4.15)
HR (bpm)	428.57	550.00[a]	550.00[a]
	(380.95-464.29)	(500.00-625.00)	(550.00-568.75)
PR (msec)	40.00	20.00[a]	40.00[b]
	(40.00-40.00)	(20.00-25.00)	(40.00-40.00)
QRS (msec)	20.00	25.00	40.00[ab]
	(20.00-20.00)	(20.00-30.00)	(37.50-40.00)
R (μvolt)	400.00	600.00[a]	650.00[a]
	(300.00-525.00)	(500.00-650.00)	(475.00-725.00)
QTo (msec)	80.00	70.00	80.00
	(80.00-80.00)	(60.00-80.00)	(60.00-80.00)
QTc (msec)	213.81	210.34	222.38
	(201.19-222.38)	(189.74-236.45)	(189.74-252.98)

a: Significance calculated by Kruskal Wallis test followed by Bonferroni adjusted pairwise comparison at $P< 0.05$ compared to control group.
b: Significance calculated by Kruskal Wallis test followed by Bonferroni adjusted pairwise comparison at $P< 0.05$ compared to hyperthyroid group.

In vitro studies on isolated perfused hearts:

I-Baseline values:

The mean ranks of baseline PT/LV (19.22, 9.20 and 17.00) and of baseline HRT 23.17, 11.95 and 10.70) for control, hyperthyroid and EVOO-treated-hyperthyroid groups. There was significant difference in the baseline values of PT/LV ($X^2(2) =7.41$, $p<0.05$) and HRT ($X^2(2)=12.17$, $p<0.005$) between the three studied groups. After Bonferroni adjusted pairwise comparison, the significant difference in baseline PT/LV was only between control group and hyperthyroid ($Z=10.02$, $p<0.05$) group. In baseline HRT significant differences were between control group and each of hyperthyroid ($Z=11.22$, $p<0.02$) and EVOO-treated-hyperthyroid ($Z=12.47$, $p<0.005$) groups. (Table 2) and (Figure 1).

II- After isoproterenol infusion:

In vitro heart rate:

For the hyperthyroid group, the HR recovery value was significantly different from the basal value ($Z=3.25$, $p<0.02$) with a mean rank of 5.60 for basal value and 2.35 for recovery value. Regarding the EVOO-treated-hyperthyroid group, both the HR response to ISO3.46 ($Z=4.45$, $p<0.001$) and recovery values ($Z=4.05$, $p<0.002$) were significantly changed from basal value with a mean rank of 6.05 for basal, 1.50 for ISO3.46 and 2.00 for recovery values. On comparing groups together, there was no significant difference in HR values. (Figure 1) and (Table 2).

Peak tension per left ventricular weight

In hyperthyroid group, the PT/LV showed significant differences between its basal value and each of ISO3.46 (Z=4.20, p<0.005) and recovery values (Z=5.40, p<0.001) with a mean rank of 7.10 for basal, 2.90 for ISO3.46 and 1.70 for recovery values. As regard the EVOO-treated-hyperthyroid group, the PT/LV response to ISO1.73 (Z=4.05, p<0.01), ISO 2.60 (Z=4.75, p<0.001), ISO3.46 (Z=5.60, p<0.001) and recovery values (Z=6.65, p<0.001) were significantly changed with a mean rank of 7.80 for basal, 3.75 for ISO1.73, 3.05 for ISO 2.60 2.20 for ISO3.46 and 1.15 for recovery values.

The mean ranks of ISO0.65 were (21.89, 9.45 and 14.35), of ISO1.3 were (24.11, 9.10 and 12.70), of ISO1.73 were (24.11, 9.90 and 11.90), of ISO2.60 were (23.83, 10.85 and 11.20), of ISO3.46 were (23.44, 11.85 and 10.55) and of the recovery values were (21.38, 10.89 and 10.90) in control, hyperthyroid and EVOO-treated-hyperthyroid groups respectively.

Comparing groups together, there were significant differences in PT/LV values responses to different ISO doses and in recovery values; ISO0.65 (X^2(2)=10.21, p<0.01), ISO1.3 (X^2(2)=15.84, p<0.001), ISO1.73 (X^2(2)=15.23, p<0.002), ISO2.60 (X^2(2)=14.06, p<0.002), ISO3.46 (X^2(2)=12.97, p<0.005) and recovery values (X^2(2)=, p<0.01) between different studied groups.

After Bonferroni adjusted pairwise comparison, significant differences were detected in ISO0.65 between control group and hyperthyroid (Z=12.44, p<0.005) group, in ISO1.3 between control group and each of hyperthyroid (Z=15.01, p<0.001) and EVOO-treated-hyperthyroid (Z=11.41, p<0.02) groups, in ISO1.73 between control group and each of hyperthyroid (Z=14.21, p<0.002) and EVOO-treated-hyperthyroid (Z=12.21, p<0.01) group, in ISO2.60 between control group and each of hyperthyroid (Z=12.98, p<0.005) and EVOO-treated-hyperthyroid (Z=12.63, p<0.005) group, in ISO3.46 were between control group and each of hyperthyroid (Z=11.59, p<0.01) and EVOO-treated-hyperthyroid (Z=12.89, p<0.005) group and in recovery values between control group and each of hyperthyroid (Z=10.49, p< 0.05) and EVOO-treated-hyperthyroid (Z=10.48, p<0.02) groups.

PT/LV maximal response showed significant difference between groups (X^2(2) =13.50, p<0.002) with mean rank of 23.44 for control, 9.65 for hyperthyroid, 12.75 for EVOO-treated-hyperthyroid groups. After Bonferroni adjusted pairwise comparison, the significant differences were between control group and each of hyperthyroid (Z=13.79, p<0.002) and EVOO-treated-hyperthyroid (Z=10.69, p<0.02) groups.

PT/LV delta change showed significant difference between groups (X^2(2)=14.30, p<0.002) with mean rank of 23.39 for control, 13.65 for hyperthyroid, 8.80 for EVOO-treated-hyperthyroid groups. After Bonferroni adjusted pairwise comparison, the significant differences were between control group and each of hyperthyroid (Z=9.74, p<0.05) and EVOO-treated-hyperthyroid (Z=14.59, p<0.002) groups. (Table 2) and (Figure 1)

Tension generation:

Compared to its basal values, dT/dt values of the control group were significantly different at ISO3.46 (Z=4.38, p<0.002) and recovery values (Z=4.25, p<0.005) with a mean rank of 6.12 for basal, 1.75 for ISO3.46 and 1.88 for recovery values. The values of dT/dt in the hyperthyroid group showed significant difference in response to ISO3.46 (Z=4.38, p<0.002) and recovery (Z=4.25, p<0.005) values with a mean rank of 6.22 for basal, 2.94 for ISO3.46 and 1.61 for recovery values. Regarding the EVOO-treated-hyperthyroid group, the response to ISO1.73 (Z=3.00, p<0.05), ISO2.60 (Z=4.10, p<0.001), ISO3.46 (Z=4.80, p<0.001) and recovery (Z=5.70, p<0.001) values with a mean rank of 6.90 for basal, 3.90 for ISO1.73, 2.80 for ISO2.60, 2.10 for ISO3.46 and 1.20 for recovery values.

The mean ranks of ISO0.65 (15.69, 7.94 and 18.10), ISO1.3 (19.12, 7.56 and 15.70), ISO1.73 (20.50,9.00 and 13.30), ISO2.60 (21.25, 10.44 and 11.40) and ISO3.46 (20.50, 10.89 and 11.60) in control, hyperthyroid and EVOO-treated-hyperthyroid groups. Comparing groups together, there were significant differences in all responses to different ISO doses; ISO0.65 (X^2(2)=8.27, p<0.02), ISO1.3 (X^2(2)=9.73, p<0.01), ISO1.73 (X^2(2)=9.01, p<0.02), ISO2.60 (X^2(2)=9.55, p<0.01) and ISO3.46 (X^2(2)=7.66, p<0.05).

After Bonferroni adjusted pairwise comparison, there was significant difference in ISO0.65 between hyperthyroid and EVOO-treated-hyperthyroid (Z= -10.16, p<0.02) groups, ISO1.3 between control and hyperthyroid (Z=11.50, p<0.01) groups, in ISO1.73 between control and hyperthyroid (Z=11.57, p<0.01) groups, in ISO2.60 between control and each of hyperthyroid (Z=10.81, p<0.02) and EVOO-treated-hyperthyroid (Z=9.85, p<0.05) and in ISO3.46between control and hyperthyroid (Z=9.61, p<0.05) groups. (Table 2) and (Figure 1).

Time to peak tension:

Regarding TPT, compared to the basal values, the response to different ISO doses in the 3 groups showed no significant difference. The mean ranks of ISO0.65 (18.88,

16.06 and 8.25), of ISO1.3 (18.75, 15.89 and 8.50) and of ISO1.73 (19.19, 14.39 and 9.50) in control, hyperthyroid and EVOO-treated-hyperthyroid groups.

Comparing groups together, there were significant differences in the responses to the first 3 ISO doses; ISO0.65 ($X^2(2)$ =8.91, p<0.02), ISO1.3 ($X^2(2)$=8.22, p<0.02) and ISO1.73 ($X^2(2)$=6.69, p<0.05) in the different studied groups.

After Bonferroni adjusted pairwise comparison, there was significant difference in ISO0.65 between control and EVOO-treated-hyperthyroid (Z=10.63, p<0.02) groups, in ISO1.3 between control and EVOO-treated-hyperthyroid (Z=10.25, p<0.02) groups and in ISO1. between control and EVOO-treated-hyperthyroid (Z=9.69, p<0.05) groups.

TPT maximal response showed significant difference between groups ($X^2(2)$ = 9.59, p<0.01) with mean rank of 19.81 for control, 15.11 for hyperthyroid, 8.35 for EVOO-treated-hyperthyroid groups. After Bonferroni adjusted pairwise comparison, the significant difference was only between control and EVOO-treated-hyperthyroid (Z=11.46, p<0.01) groups. (Table 2) and (Figure 1).

Half relaxation time:

For the HRT, compared to the basal values, the hyperthyroid group showed significant difference in the recovery value (Z= -3.25, p<0.02) with mean rank of 2.45 for basal and 5.70 for recovery values. For the EVOO-treated-hyperthyroid group, there was significant difference between its basal and each of response to ISO3.46 (Z= -3.45, p<0.01) and recovery value (Z= -4.15, p<0.001) with mean rank of 2.65 for basal, 6.10 for ISO3.46, 6.80 for recovery values. Comparing groups together, there was significant difference between the 3 groups in response to ISO0.65 ($X^2(2)$ =7.99, p<0.02) with mean rank of 21.44 for control, 13.35 for hyperthyroid and 10.85 for EVOO-treated-hyperthyroid group. After Bonferroni adjusted pairwise comparison, the significant difference was only between control and EVOO-treated-hyperthyroid (Z=10.59, p<0.02) groups. (Table 2) and (Figure 1).

Myocardial flow rate per left ventricular weight:

Finally, MFR/LV, compared to the basal values, the control group showed significant difference in the response to ISO3.46 and recovery value (Z=4.71, p<0.002 for both) with the mean rank of 6.50 for basal, 1.79 for both ISO3.46 and recovery values. The hyperthyroid group showed significant difference in the response to ISO2.60 (Z=3.67, p<0.01), ISO3.46 (Z=5.00, p<0.001) and recovery value (Z=5.72, p<0.001) with mean rank of 7.00 for basal, 3.33 for ISO2.60, 2.00 for ISO3.46 and 1.28 for recovery values. For the EVOO-treated-hyperthyroid group, there were significant differences between its basal and each of response to ISO2.60 (Z=4.00, p<0.002), ISO3.46 (Z=5.10, p<0.001) and recovery value (Z=5.90, p<0.001) with mean rank of 7.00 for basal, 3.00 for ISO2,60, 1.90 for ISO3.46, 1.10 for recovery values. Comparing groups together, there was no significant difference between the 3 groups. (Table 2) and (Figure 1).

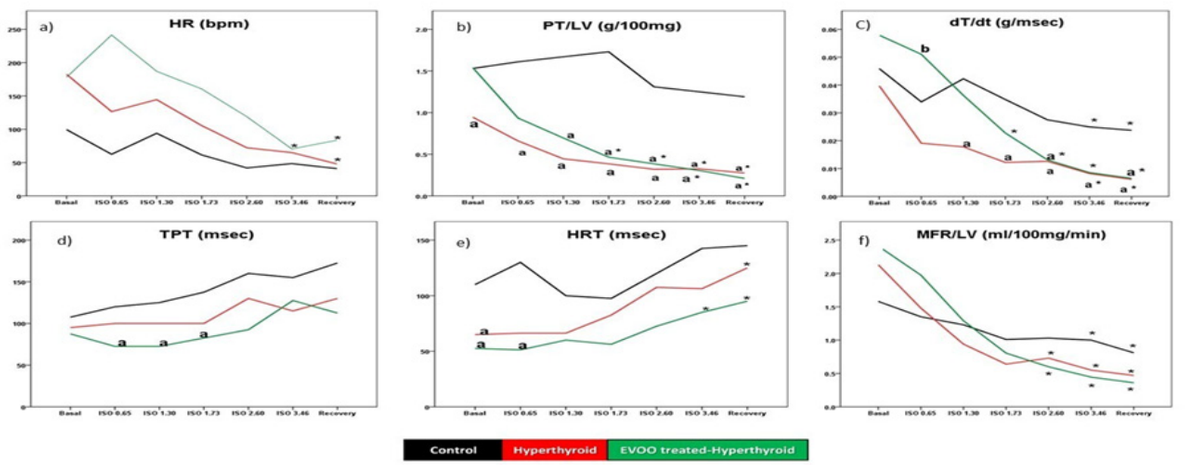

Fig. 1: Isolated heart study: The baseline, graded response to isoproterenol infusion (0.65, 1.3, 1.73, 1.6 and 3.46 µg/min for 3 min) and recovery Median values of a) Heart beating rate (HR), b) peak developed tension per left ventricular weight (PT/LV), c) rate of tension development (Td/td), d) time to peak tension (TPT), e) half relaxation time (HRT) and f) and myocardial flow rate per left ventricular weight (MFR/LV) in the from the different studied groups.
a: Significance calculated by Kruskal Wallis test followed by Bonferroni adjusted pairwise comparison at P< 0.05 compared to control group.
b: Significance calculated by Kruskal Wallis test followed by Bonferroni adjusted pairwise comparison at P< 0.05 compared to hyperthyroid group.
*: Significant calculated by Friedman's test followed by Bonferroni adjusted pairwise comparison at P< 0.05 compared to baseline value.

Table 2: Median and interquartile range (IQR) of heart beating rate (HR), peak developed tension per left ventricular weight (PT/LV), rate of tension development (dT/dt), time to peak tension (TPT), half relaxation time (HRT), and myocardial flow rate per left ventricular weight (MFR/LV); at baseline, maximal responses to isoproterenol, and delta changes of perfused hearts isolated from all studied groups.

	Baseline			Maximal responses			Delta changes		
	Control	Hyperthyroid	EVOO-treated-Hyperthyroid	Control	Hyperthyroid	EVOO-treated-Hyperthyroid	Control	Hyperthyroid	EVOO-treated-Hyperthyroid
HR (bpm)	99.50 (52.25-159.50)	182.50 (140.25-245.00)	178.00 (152.50-295.50)	94.00 (53.50-206.50)	183.00 (132.75-283.50)	241.50 (147.25-285.00)	15.50 (-13.25-50.50)	0.50 (-32.50-55.00)	5.00 (-29.00-64.00)
PT/LV (g/100 mg)	1.53 (1.32-1.72)	0.95[a] (0.67-1.20)	1.54 (0.89-1.90)	2.02 (1.52-2.51)	0.79[a] (0.37-1.03)	0.94[a] (0.70-1.48)	0.64 (0.06-0.91)	-0.15[a] (-0.36- 0.05)	-0.47[a] (-0.62- -0.08)
dT/dt (g/msec)	0.05 (0.03-0.06)	0.04 (0.03-0.05)	0.06 (0.04-0.09)	0.04 (0.03-0.05)	0.023 (0.015-0.039)	0.05 (0.03-0.08)	-0.004 (-0.01-0.02)	-0.008 (-0.021-0.008)	-0.02[b] (-0.02- -0.01)
TPT (msec)	107.50 (88.75-115.00)	95.00 (77.50-112.50)	87.50 (70.00-95.00)	112.50 (80.00-137.50)	80.00 (70.00-107.50)	65.00 a (53.75-78.75)	2.50 (-22.50-32.50)	-15.00 (-40.00-22.50)	-17.50 (-26.25- -3.75)
HRT (msec)	110.00 (91.25-136.25)	65.00[a] (50.00-73.75)	52.50[a] (50.00-70.00)	97.50 (50.00-156.25)	47.50 (41.88-83.13)	50.00 (48.13-58.75)	-22.50 (-41.25-37.50)	-7.50 (-13.75-11.25)	-2.50 (-7.50- -1.88)
MFR/LV (ml /100mg/ min)	1.58 (1.31-2.47)	2.13 (1.29-2.35)	2.41 (1.47-2.97)	1.40 (1.04-2.12)	1.48 (0.96-1.98)	1.98 (1.39-2.37)	-0.35 (-0.62-0.00)	-0.44 (-0.56- -0.17)	-0.34 (-0.61- -0.20)

a: Significance calculated by Kruskal Wallis test followed by Bonferroni adjusted pairwise comparison at $P< 0.05$ compared to control group.
b: Significance calculated by Kruskal Wallis test followed by Bonferroni adjusted pairwise comparison at $P< 0.05$ compared to hyperthyroid group.

Biochemical measurements:

There was statistically significant difference in serum free T3 ($X^2(2)=13.43$, $p<0.002$) between the three studied groups, with a mean rank of 6.44 for control group, 19.80 for hyperthyroid group and 17.90 for EVOO-treated-hyperthyroid group. After Bonferroni adjusted pairwise comparison, the significant differences were only between control group and each of hyperthyroid ($Z= -13.36$, $p<0.005$), EVOO-treated-hyperthyroid ($Z=-11.46$, $p<0.02$) groups. Serum free T4 was significantly increased while serum TSH was significantly decreased in both hyperthyroid and EVOO-treated-hyperthyroid groups as compared to control rats. Cardiac tissue HO-1 was significantly increased in hyperthyroid group as compared to control group, on EVOO treatment HO-1 was significantly decreased as compared to hyperthyroid group being comparable from controls. (Table 3).

Table 3: Median and interquartile range (IQR) of serum tri-iodothyronin (T3), Mean± SD of tetra-iodothyronin (T4) and thyroid stimulating hormone (TSH) and cardiac tissue hemeoxygenase 1 (HO-1) levels in all studied groups.

	Control	Hyperthyroid	EVOO-treated-Hyperthyroid
T3 (pg/ml)	6.80 (4.90-7.95)	16.90[á] (9.38-19.73)	14.60[á] (9.80-18.15)
T4 (ng/dl)	2.19±0.56	6.98±2.43[a]	7.08±1.59[a]
TSH (ulU/ml)	0.01±0.005	0.01±0.00[a]	0.01±0.00[a]
Cardiac HO-1 (ng/mg)	3.06±0.81	15.25±9.72[a]	8.08±2.45[b]

In Parametric data
a: Significance calculated by One-way ANOVA followed by Post Hoc Bonferroni test from control group.
b: Significance calculated by One-way ANOVA followed by Post Hoc Bonferroni test from hyperthyroid group.
á: Significance calculated by Kruskal Wallis test followed by Bonferroni adjusted pairwise comparison compared to control group.

Correlation studies:

Serum T3 showed significant positive correlation with cardiac hemeoxygenase-1, relative left ventricular weight, R wave voltage and in vivo heart rate, while it showed significant negative correlation with PR interval, peak tension per left ventricle after last dose of isoproterenol infusion.

Cardiac angiotensin II receptors mean area % and optical density either before being subjected to isolated heart study or after isolated heart study showed significant positive correlation with serum T3, cardiac hemeoxygenase-1, relative left ventricular weight, R voltage and in vivo heart rate, while they showed significant negative correlation with PR interval, basal peak tension per left ventricle, peak tension per left ventricle after infusion of isoproterenol last dose

Cardiac hemeoxygenase-1 showed significant positive correlation with relative left ventricular weight, R wave voltage and in vivo heart rate, while it showed significant negative correlation PR interval, peak tension per left ventricle after infusion of isoproterenol last dose and tension generation after infusion of isoproterenol last dose. (Table 4).

Table 4: Spearman correlation of serum T3, cardiac angiotensin II receptor mean area %, optical density and cardiac hemoxygenase-1 levels with different parameters.

			Cardiac Ang-II R				
		Serum T3	Not subjected to isolated heart study		Subjected to isolated heart study		Cardiac HO1
			Mean area %	OD	Mean area %	OD	
Serum T3	rs	-	0.605	0.412	0.641	0.497	0.697
	P	-	≤0.001	≤0.05	≤0.001	≤0.01	≤0.001
Cardiac HO1	rs	0.697	0.676	0.622	0.724	0.688	--
	p	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	
LV/BW	rs	0.441	0.420	0.526	0.540	0.513	0.594
	P	≤0.05	≤0.05	≤0.01	≤0.01	≤0.01	≤0.001
R voltage	rs	0.425	0.390	0.502	0.531	0.455	0.477
	P	≤0.05	≤0.05	≤0.01	≤0.01	≤0.05	≤0.01
HR	rs	0.591	0.550	0.482	0.676	0.452	0.634
	P	≤0.001	≤0.01	≤0.01	≤0.001	≤0.05	≤0.001
PR interval duration	rs	-0.367	-0.729	-0.610	-0.634	-0.618	-0.557
	P	≤0.05	≤0.001	≤0.001	≤0.001	≤0.001	≤0.01
Basal PT/LV	rs	-0.284	-0.444	-0.587	-0.494	-0.607	-0.302
	P	0.135	≤0.05	≤0.001	≤0.01	≤0.001	0.118
ISO last dose PT/LV	rs	-0.461	-0.541	-0.551	-0.504	-0.643	-0.589
	P	≤0.05	≤0.01	≤0.01	≤0.01	≤0.001	≤0.001
ISO last dose dT/dt	rs	-0.286	-0.457	-0.432	-0.570	-0.440	-0.390
	P	0.149	≤0.05	≤0.05	≤0.01	≤0.05	≤0.05

rs: Spearman rank correlation coefficient.
P: Significance level of spearman rank correlation.

Histopathological examination of left ventricular specimens:

Hematoxylin and Eosin (H&E) stained sections:

- Not subjected to isolated heart study: Examination of the H&E-stained left ventricle specimens from rats not subjected to the isolated heart study of the control group revealed regularly arranged cardiac muscle fibers, appearing branching anastomosing and running in various directions. The cardiomyocytes appeared striated and showed central vesicular nuclei and acidophilic sarcoplasm. In transverse section, the cardiac myocytes appeared more or less comparable in size with noticeable myofibrillar content (Figure 2a). Cardiac specimens from the hyperthyroid group revealed apparent structural changes in the cardiac muscle fibers. Many fibers appeared irregularly arranged, widely separated and discontinuous in many areas. Some fibers appeared enlarged with deep acidophilic cytoplasm and pyknotic nuclei. Mononuclear cellular infiltration and congestion was also detected in this group (Figure 2b,c). Examination of the cardiac muscle fibers of the EVOO-treated hyperthyroid group revealed branching and anastomosing fibers. The cardiac muscle fibers appeared similar to the control group being acidophilic with central vesicular nuclei, with minimal mononuclear infiltration (Figure 2d).

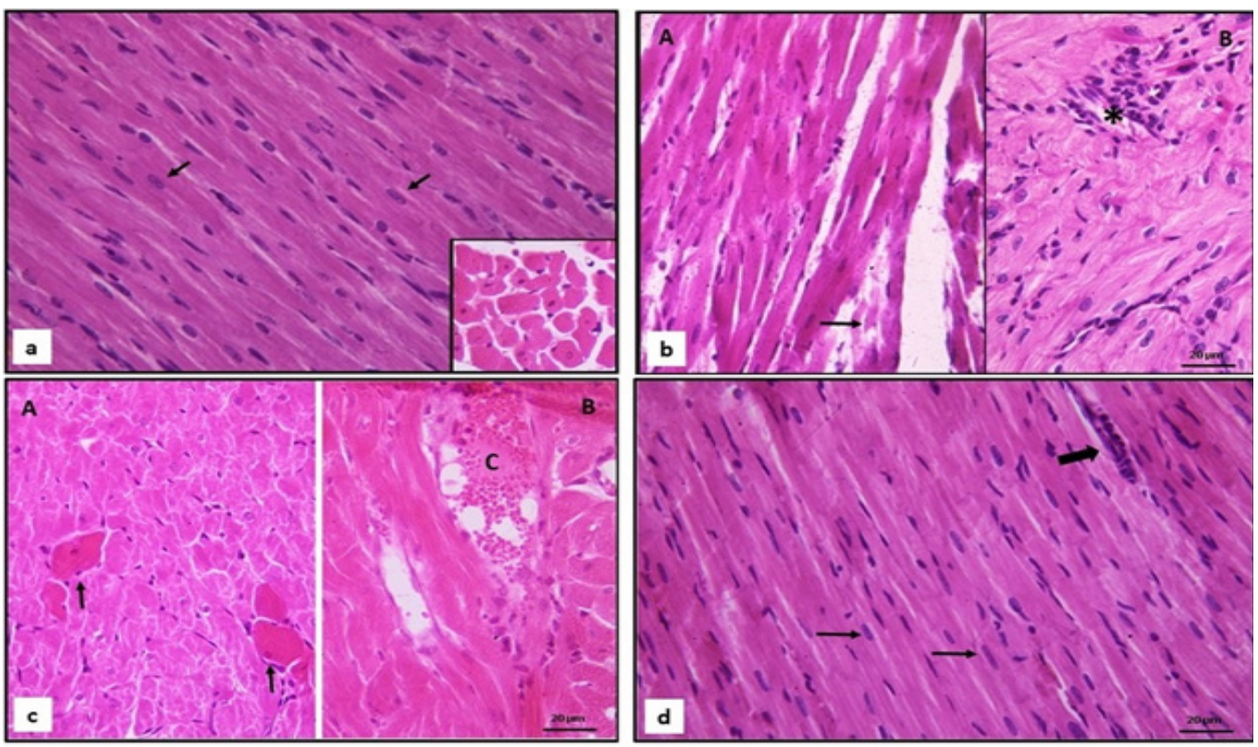

Fig. 2: Photomicrograph of H&E-stained heart sections in rats non subjected to isolated heart study. (a) Control group: Showing regularly arranged cardiac muscle fibers. They show acidophilic cytoplasm and central vesicular nuclei (↑). Inset: Showing transverse section of cardiac muscle fibers uniform in size. (b) Hyperthyroid group: showing an area of widely separated discontinuous (↑) cardiac muscle fibers (A), mononuclear cellular infiltration can be seen (*). (c) Hyperthyroid group: Showing enlarged cardiomyocytes with deep acidophilic cytoplasm and pyknotic nuclei (↑) in between regular sized cardiomyocytes (A) and area of congestion (C) is detected (B). (d) EVOO-treated hyperthyroid group: Showing cardiomyocytes regularly arranged with vesicular nuclei (↑) and acidophilic cytoplasm, minimal mononuclear infiltration can be detected (thick arrow). (H&E, X 400)

- **Subjected to isolated heart study:**

Examination after the isolated heart study, following rat exposure to isoproterenol infusion the control group revealed moderate distortion of the cardiomyocytes. They appeared branching and anastomosing with focal separation and discontinuity between the fibers. Some fibers showed deep acidophilic cytoplasm (Figure 3a). Cardiac specimen from hyperthyroid group revealed marked affection of the left ventricle. The cardiomyocytes appeared irregularly arranged with marked separation. The fibers showed marked vacuolation and some fibers appeared apparently enlarged, deeply acidophilic with pyknotic nuclei. Mononuclear cellular infiltration and congestion were detected in these specimens (Figure 3 b,c,d). EVOO-treated hyperthyroid group showed some fibers similar to the control group while others were affected. Focal separation between the fibers was detected. Moderate congestion was also detected in this group (Figure 3 e,f).

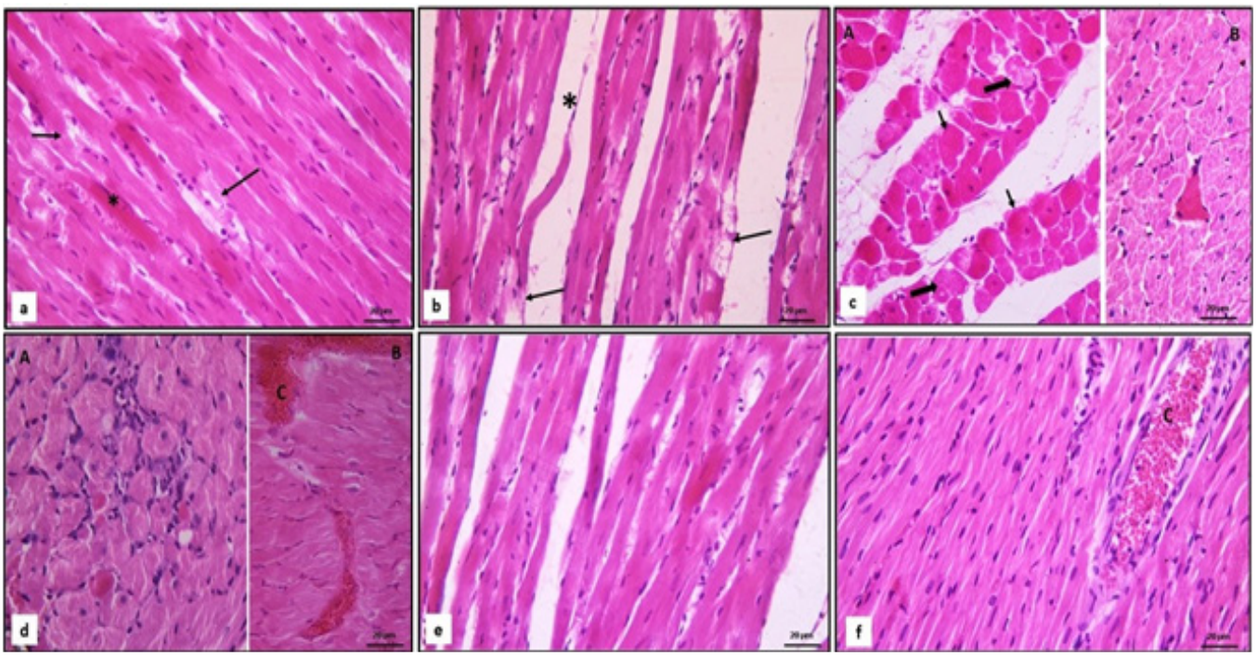

Fig. 3: Photomicrograph of H&E-stained the cardiac muscle fibers in rats subjected to isolated heart study:
(a) Control group: Showing focal interrupted and discontinuous (↑) cardiac muscle fibers. Notice some fibers show deep acidophilic cytoplasm (*). (b) Hyperthyroid group: Showing widely separated (*) cardiomyocytes. Some fibers show cytoplasmic vacuolation (↑). (c) Hyperthyroid group: Showing an area of enlarged deep acidophilic cardiomyocytes with shrunken pyknotic nuclei (↑). Some fibers show vacuolated (thick arrow) cytoplasm (A). A large deep acidophilic cardiomyocyte with a small pyknotic nucleus is seen between regular sized cardiac muscle fibers (B). (d) Hyperthyroid group: Showed an area of mononuclear cellular infiltration (A) and an area of congestion (C) between the cardiomyocytes (B). (e) EVOO-treated hyperthyroid group: Showed widely separated cardiomyocytes with acidophilic cytoplasm. (f) EVOO-treated hyperthyroid group: Showed marked congestion of blood vessel between the cardiomyocytes (C). (H&E, X 400).

Angiotensin II receptor1 immunohistochemistry:

- Not subjected to isolated heart study: Examination of cardiac specimens from the control group showed weak positive reaction for angiotensin II receptor which appeared brown in color with little distribution (Figure 4a). The hyperthyroid group showed a widespread distribution of a moderate positive reaction (Figure 4b). In the EVOO-treated hyperthyroid group, the reaction was weak and similar to the control group (Figure 4c).
- Subjected to isolated heart study: Cardiac specimens from the control group after isoproterenol infusion showed weak positive reaction for angiotensin II receptor with little distribution (Figure 4d). The hyperthyroid group showed widely distributed extensive strong positive reaction for angiotensin II (Figure 4e). The EVOO-treated hyperthyroid group showed moderate positive reaction (Figure 4f).

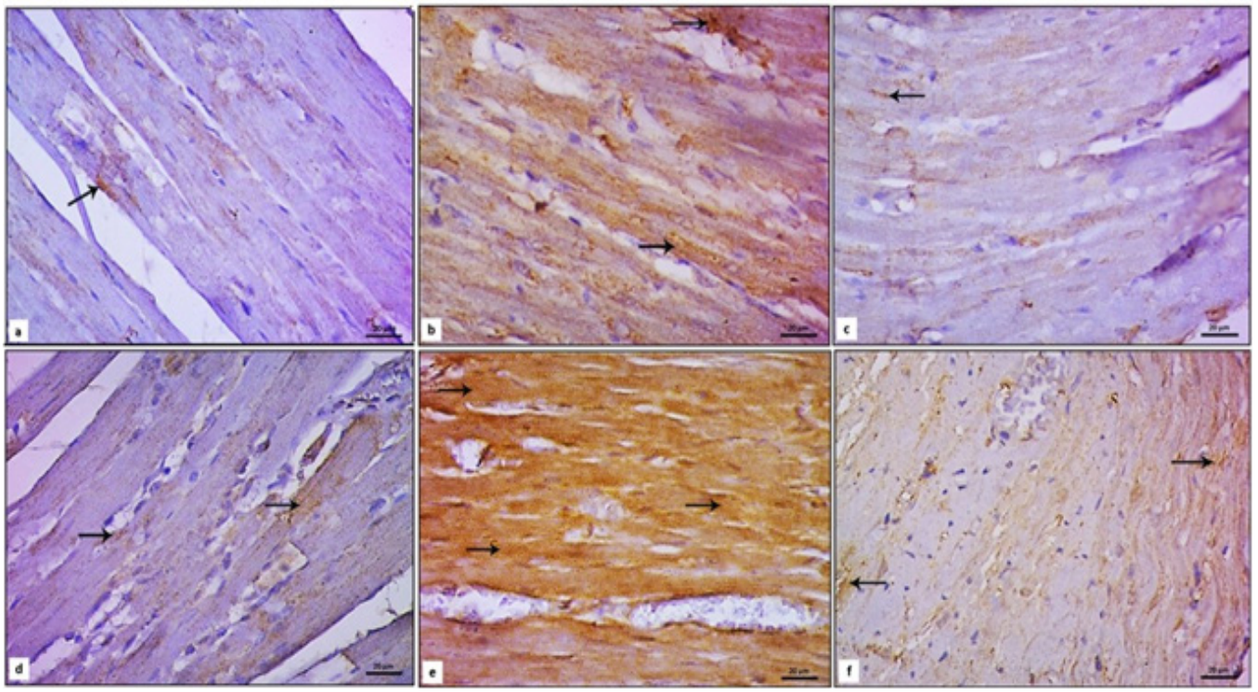

Fig. 4: Immunohistochemistry of Angiotensin II receptor in the cardiac muscle fibers:
In rats not subjected to isolated heart study (a) Control group: Showed weak positive reaction. (b) Hyperthyroid group: Showed widespread moderate positive reaction. (c) EVOO-treated hyperthyroid group: Showed weak positive reaction. In rats subjected to in isolated heart study (d) Control group: Showed weak positive reaction. (e) Hyperthyroid group: Showed widespread strong positive reaction. (f) EVOO-treated hyperthyroid group: Showed moderate positive reaction. (Streptavidin-biotin peroxidase, X400)

Histomorphometric analysis:

- Not subjected to isolated heart study: The mean area % and mean optical density of angiotensin II receptor were significantly increased in the hyperthyroid group as compared to its counter control group. On the other hand, the EVOO-treated hyperthyroid group showed a significant decrease as compared to the hyperthyroid group and a non-significant increase as compared to the control group (Table 5).
- Subjected to isolated heart study: Both the mean area % and mean optical density of angiotensin II receptor were significantly increased in the hyperthyroid and the EVOO-treated hyperthyroid groups as compared to their counter control group. On the other hand, the EVOO-treated hyperthyroid group showed a significant decrease as compared to the hyperthyroid group (Table 5).

Table 5: Mean± SD of mean area percentage (%) and optical density (O.D.) of cardiac angiotensin (Ang) II receptor in rats both not subjected and subjected to isolated heart study in all studied groups.

		Control	Hyperthyroid	EVOO-treated-Hyperthyroid
Not subjected	Mean area %	1.43 ± 0.11	16.95 ± 2.41[a]	1.74 ± 0.14[b]
	O.D.	58.36±4.05	72.63±2.80[a]	61.43±2.38[b]
Subjected	Mean area %	3.23±0.52	33.65±4.45[a]	6.28±0.23[ab]
	O.D.	62.40±7.29	82.87±7.81[a]	73.17±5.75[ab]

a: Significance calculated by One-way ANOVA followed by Post Hoc Bonferroni test at $P<0.05$ from control group. b: Significance calculated by One-way ANOVA followed by Post Hoc Bonferroni test at $P<0.05$ from hyperthyroid group.

DISCUSSION

This study portrayed the possible cardioprotective effects of EVOO supplementation in experimentally induced hyperthyroidism rat model, through assessment of ECG changes, cardiac intrinsic properties and their responses to isoproterenol infusion as a beta agonist. Also, the study highlighted the role of angiotensin II receptor1 expression in mediating these effects.

Hyperthyroid state was established after administration of 100µg/kg L-thyroxine evidenced by significant elevation in serum free T3, T4 accompanied by significant decrease in serum TSH, % BW and %BMI compared to the control group. The noticed weight reduction is a primary sign of hyperthyroidism owing to the increased basal metabolic rate and oxygen consumption in tissues[22]. Olive oil supplementation increased weight loss due to the added inhibitory effect on lipid formation exerted by its polyphenolic compounds[23].

The significant body weight reduction added to the significant increase in absolute ventricular weights resulted in significant increased relative ventricular weights in hyperthyroid groups. This denoted cardiac hypertrophy similar to previous earlier reports[24], which might result from either left ventricular loading consequent to the adrenergic nervous system stimulation by thyroid hormone[25] or due to a direct response of thyroid hormone supported by the positive correlation between T3 and relative left ventricular weight. This could be related to thyroid hormone ability to increase rates of protein synthesis, upregulate the gene expression of several cell proliferating signaling pathways[26].

Also, it might be related to the close relationship between thyroid hormones and the renin-angiotensin system owing to the proposed role of angiotensin II as a growth factor that can induce cardiac growth and hypertrophy through angiotensin II receptors leading to the activation of the mitogen activated protein kinases (MAPK)[27].

The significant increase in area percentage and optical density of cardiac angiotensin II receptors in immunohistochemical studies of hyperthyroid group added to their positive correlation with serum T3 and left ventricular weight advocates this assumption. This goes with previous reports which suggested that thyroid hormone may increase the angiotensin II receptors expression, enhance the cardiac expression of renin mRNA, leading to increased cardiac levels of renin and angiotensin II[28]. In the same context, previous studies by ***Kim et al.***[29] stated that angiotensin receptor type I blockers were more effective than β-blockers in improving cardiac hypertrophy induced by hyperthyroidism, highlighting angiotensin role.

Further, Ang II-induced cardiac hypertrophy may be mediated through augmenting reactive oxygen species production[30], which play an important role in mediating hypertrophy through promoting myocardial growth, extracellular matrix remodeling by activating hypertrophy signaling kinases and transcription factors[31]. The elevated oxidative state in the hyperthyroid group was reflected by the significant increase of HO-1 being an antioxidative defense mechanism, with a positive correlation detected between HO-1 and both serum T3, cardiac angiotensin II receptors. Also, cardiac HO-1 was positively correlated with relative left ventricular weight. This was in accordance with earlier study by ***Ruiz-Ortega et al.***[32] who claimed that Ang II up-regulates HO-1 a stress-responsive protein, as an adaptive response to oxidative injury. In addition, ***Campbell et al.***[33] stated that HO-1 is known to be upregulated by most cells in response to pro-oxidant stimuli to provide protection against oxidative damage.

Also, ***Li et al.***[34] stated that T3 upregulate HO-1 expression accompanied with transient induction of oxidative stress to offer cytoprotection through its antioxidant function. ***Ha et al.***,[35] explained that the activated β1-adrenoreceptors in hyperthyroid state might upregulate HO-1 gene expression through stimulating cyclic adenosine monophosphate which activates p38 MAPK activity and nuclear factor-erythroid-related factor 2.

EVOO supplementation was able to decrease area percentage and optical density of angiotensin II receptors in immunohistochemical studies which was accompanied by improvement of the oxidative state evidenced the consequent significant reduction in HO-1 enzyme. Similarly, ***Araujo et al.***[36] stated that the increase in HO-1 several folds reflected cardiac injury by different inflammatory stressors after transplantation.

In line with this study, ***Martin-Pelaez et al.***[37] reports that olive oil might modulate the expression of some of the genes related to the renin-angiotensin-aldosterone system owing to its phenolic compounds. In addition, ***Vazquez et al.***[38] detected significant decrease in serum angiotensin II in rats following olive oil treatment. This improved oxidative state could also result from the direct activity of EVOO phenolic compounds as hydroxy tyrosol and secoiridoids[39]. This could explain the partial attenuation of ventricular hypertrophy in olive oil-treated hyperthyroid group indicated by lower absolute left ventricular weight than hyperthyroid group. Meanwhile absolute left ventricular weight was still higher than that of the control denoting that given olive oil dose was not able to fully revert

cardiac hypertrophy owing to their inability to combat the adrenergic effect of the high thyroid hormones. Also, relative left ventricular or whole heart weights were non-significantly changed from the hyperthyroid group which can be related to the significant body weight reduction in this group.

In line with the recorded ventricular hypertrophy, QRS duration and R wave were significantly increased in hyperthyroid-treated and EVOO-treated groups. Increased sympathetic activity in hyperthyroid group was associated with significant increase in HR and shortening in PR interval, which showed a positive correlation and negative correlation with thyroid hormone respectively. Thyroid hormone can increase the expression of sinus node L-type calcium channel, activate hyperpolarization cyclic nucleotide channels enhancing pacemaker potential[40]. Also, enhanced conduction through myocardial gap junctions might explain short PR interval owing to connexin-40 mRNA upregulation due to direct action of thyroid hormone[41], consequent to increased angiotensin II[42] or due to the associated oxidative stress[43]. Olive oil antioxidant activity and decreased angiotensin II receptor expression might explain its ability to restore PR interval to its normal level.

The in-vitro recording matches with the in-vivo recorded tachycardia, suggesting the direct effect of thyroid hormone on sinus node ion channels, myocardial transporters and exchangers that regulate cellular excitability[44]. Meanwhile, heart rate delta change was lower than control, suggesting lower cardiac reserve and inability to further increase heart rate. In olive oil supplemented group, although basal heart rate was still higher than control, delta change of heart rate in response to isoproterenol infusion was improved.

In vitro studies revealed deterioration in the inotropic activity in hyperthyroid group, proved by the significant decrease in the basal PT/LV, non-significant decrease in basal dT/dt time compared to the control group. This deterioration was augmented following isoproterenol infusion with lower delta PT/LV and dT/dt values denoting early toxicity following adrenergic stimulation. In spite of the significant shortening of basal HRT, following isoproterenol infusion HRT delta change was lower than that of the control.

Systolic and diastolic cardiac dysfunction could be attributed to increased tissue sensitivity to catecholamines in hyperthyroidism which might hyper-phosphorylate Ryanodine Receptor 2 (RyR2) the calcium release channel or displace calstabin, the calcium channel stabilizer, from RyR2, altering the gating of the channel, which results in Ca2+ leakage[28].

Calcium overload also might result from the associated tachycardia, enhanced oxidative stress or the elevated angiotensin receptors expression which showed significant elevation after isoproterenol infusion. In the same context, ***Bhullar et al.***,[45] stated that Ang-II-R1-mediated intracellular signal transductions contribute to progression of cardiac remodeling and cardiomyocyte calcium overload. Cardiomyocyte Ca2+ -overload produces mitochondrial Ca2+-overload with alterations in electron transport chain, generation of reactive oxygen species triggering the opening of mitochondrial permeability transition Pore with consequent depression in ATP production, metabolic derangements that end with cardiomyocyte apoptosis and cellular damage[46]. This was supported by the significant negative correlation between angiotensin II receptor expression and basal peak tension per left ventricle, peak tension per left ventricle and

This was reflected in histological examination that showed irregularly arranged, widely separated and discontinuous cardiac muscle fibers with pyknotic nuclei denoting damaged myocardial cells. Therefore, thyroid hormones induced cardiac hypertrophy, remodeling, enhanced angiotensin II sensitivity with consequent elevated oxidative stress might overcome their ability to enhance sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA) leading to cardiac dysfunction[47].

EVOO treatment imparted partial mitigation in cardiac intrinsic properties. It increased basal PT/LV and dT/dt being non-significant from the control, but it didn't improve the delta changes following isoproterenol infusion. Histological examination revealed branching and anastomosing cardiac muscle fibers similar to the control group being acidophilic with vesicular nuclei. Cardiac hemeoxygenase-1 levels showed significant negative correlation with basal PT/LV reflecting that the improved oxidative and inflammatory states deduced from lower hemeoxygenase-1 levels could be linked to the improvement in cardiac systolic properties.

In the same context, olive oil was able to improve cardiac function in spontaneously hypertensive rats[38] and in myocardial infarction model[48]. This improvement could be attributed to the ability of hydroxy tyrosol a major polyphenol and one of the main constitutes of olive oil to promote antioxidant genes as catalase or thioredoxin reductase[49].

Further, olive oil ability to decrease angiotensin II receptors expression as was shown in immunohistochemistry might hinder the effects of angiotensin II. Angiotensin II up-regulates NADPH oxidase and down-regulates antioxidants, increasing oxidative stress in a positive

feedback manner leading to mitochondrial damage, endoplasmic reticulum stress, deoxyribonucleic acid damage and eventual cell death[50].

The increase in myocardial flow rate in hyperthyroid and olive oil groups could be explained by the increased cardiac work and oxygen consumption[51]. Meanwhile, following beta agonist injection, delta change coronary flow in olive oil treated rats was similar to control. This could be explained by the ability of oleic acid a major constituent of olive oil to inhibit the proliferation of vascular smooth muscles induced by angiotensin II[52], inhibit inflammatory effect of nuclear factor kappa B, alleviate oxidative stress which will mitigate endothelial dysfunction[50].

CONCLUSION

Therefore, it was concluded that EVOO was able to impart partial protection on cardiac dysfunction in hyperthyroid states as it improved some ECG parameters, basal cardiac inotropy, cardiac responses to isoproterenol and myocardial flow rate. This effect could be related to the ability of EVOO to decrease angiotensin II receptors percentage in immunohistochemistry with consequent mitigation in oxidative state evidenced by the decrease in cardiac heme-oxygenase 1.

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AUTHORS CONTRIBUTION

The study design was made by N.S., A.A., M.S. and D.A. E.M. and N.S. were a major contributor to the practical steps of the experiment. HS performed the histological examination. All authors analyzed and interpreted the results, contributed to the writing of the manuscript, read and approved the final manuscript.

CONFLICT OF INTERESTS

There is no conflicts of interest.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

REFERENCES

1. **Grais IM, Sowers JR (2014):** Thyroid and the heart. The American journal of medicine.; 127:691-698. DOI 10.1016/j.amjmed.2014.03.009

2. **Osuna PM, Udovcic M, Sharma MD (2017):** Hyperthyroidism and the heart. Methodist DeBakey Cardiololovascular journal.; 13(2):60–63. DOI 10.14797/mdcj-13-2-60.

3. **Jeppesen PL, Christensen GL, Schneider M, Nossent AY, Jensen HB, Andersen DC, Eskildsen T, Gammeltoft S, Hansen JL, Sheikh SP. (2011):** Angiotensin II type 1 receptor signaling regulates microRNA differentially in cardiac fibroblasts and myocytes. British Journal of Pharmacology. 164(2): 394-404. doi: 10.1111/j.1476-5381. 01375.x

4. **Diniz GP, Carneiro-Ramos MS, Barreto-Chaves ML. (2009):** Angiotensin type 1 receptor mediates thyroid hormone-induced cardiomyocyte hypertrophy through the Akt/GSK-3beta/mTOR signaling pathway. Basic Res Cardiol.;104(6):653-67. doi: 10.1007/ s00395-009-0043-1.

5. **Araujo A, Diniz G, Seibel F, Branchini G, Ribeiro M, Brum IS, Khaper N, Barreto-Chaves MLM, Belleó-Klein (2011):** Reactive oxygen and nitrogen species balance in the determination of thyroid hormones-induced cardiac hypertrophy mediated by renin–angiotensin system. Mol. Cell. Endocrinology. 333:78-84. Doi.org/10.1016/j.mce.2010.12.018

6. **Barreto-Chaves ML, Carrillo-Sepúlveda MA, Carneiro-Ramos MS, Gomes DA, Diniz GP (2010):** The crosstalk between thyroid hormones and the Renin-Angiotensin System. Vascular Pharmacology.52(3-4):166-170. doi: 10.1016/j. vph.2009.10.009.

7. **Guasch-Ferré M, Li Y, Willett WC, Sun Q, Sampson L, Salas-Salvadó J, Martínez-González MA, Stampfer MJ, Hu FB. (2022):** Consumption of Olive Oil and Risk of Total and Cause-Specific Mortality Among U.S. Adults. J Am Coll Cardiol. 79(2):101-112. doi: 10.1016/j.jacc.2021.10.041.

8. **Baccouri B, Sieren T, Mohamed SM, Willenberg I. (2023):** Fingerprinting of tocopherol, phenolic compounds and oxidative properties of unstudied minor and rare Tunisian olive oils. South African Journal of Botany.156:54-64. DOI:10.1016/j. sajb.2023.03.003

9. **Tanideh, N., Habibagahi, R., Ghafari, V., Ebrahimi, R., Koohi-Hosseinabadi, O., & Iraji, A. (2020):** Effects of Calendula Officinalis and Hypericum Perforatum on antioxidant, anti-inflammatory and histopathology indices of induced periodontitis in male rats. Journal of Dentistry.21(4): 1–8. Doi: 10.30476/ DENTJODS.2020.83660.1056

10. **González-Hedström D, Amor S, de la Fuente-Fernández M, Tejera-Muñoz A, Priego T, Martín, A I, López-Calderón A, Inarejos-García A M, García-Villalón Á L. Granado M. (2020):** A Mixture of Algae and Extra Virgin Olive Oils Attenuates the Cardiometabolic Alterations Associated with Aging in Male Wistar Rats. Antioxidants (Basel, Switzerland).9(6): 483. Doi: 10.3390/antiox9060483.

11. **Alcaide-Hidalgo JM, Margalef M, Bravo FI, Muguerza B, López-Huertas E. (2020):** Virgin olive oil (unfiltered) extract contains peptides and possesses ACE inhibitory and antihypertensive activity. Clinical nutrition.39(4):1242-1249. https://doi.org/10.1016/j. clnu.2019.05.016

12. **Domínguez-Vías G, Segarra AB, Ramírez-Sánchez M, Prieto I. (2021)** Effects of Virgin Olive Oil on Blood Pressure and Renal Aminopeptidase Activities in Male Wistar Rats. Int J Mol Sci. ;22(10):5388. doi: 10.3390/ijms22105388.

13. **Ryter SW, Alam J, Choi AM. (2006):** Heme oxygenase 1/carbon monoxide: from basic science to therapeutic applications. Physiology Reviews.86:583-650. Doi: 10.1152/physrev.00011.2005.

14. **Hu C M, Chen Y H, Chiang M T, Chau L Y. (2004):** Heme oxygenase-1 inhibits angiotensin II-induced cardiac hypertrophy in vitro and in vivo. Circulation.110(3), 309–316. Doi: 10.1161/01. CIR. 0000135475. 35758.23.

15. **Zrelli H, Kusunoki M, Miyazaki H. (2015):** Role of Hydroxytyrosol-dependent Regulation of HO-1 Expression in Promoting Wound Healing of Vascular Endothelial Cells via Nrf2 De Novo Synthesis and Stabilization. Phytother Res. ;29(7):1011-8. doi: 10.1002/ptr.5339.

16. **Pakdel R, Niazmand S, Mouhebati M, Vahedi M M, Aghaee A and Hadjzadeh MA (2018):** A comparison between the effects of Portulaca oleracea seeds extract and valsartan on echocardiographic and hemodynamic parameters in rats with levothyroxine-induced thyrotoxicosis. Australian Journal of Pharmacy. 8(3):276-285. PMID: 29881713; PMCID: PMC5987442.

17. **Saleh NK, Saleh HA. (2010):** Olive oil improved the impairment of in vitro insulin-stimulated glucose uptake by diaphragm in ovariectomized female Wistar rats. Experimental Gerontology.45:964–969. doi: 10.1016/j. exger.2010.08.015.

18. **Ayobe MH, Tarazi RC. (1983):** Beta receptors and contractile reserve in left ventricular hypertrophy. Hypertension. 5:192-197. Doi.org/ 10.1161/ 01. HYP.5.2_Pt_2.I192

19. **Bancroft JD, Gamble M. (2002):** Theory and Practice of Histological Techniques. Fifth edition. London U.K, Churchill Livingstone. P 377-694.

20. **Suvarna K. S., Layton C., and Bancroft J. D. eds. (2019),** Bancroft's theory and practice of histological techniques. 8th edition. Oxford: Elsevier Health Science, p. 456.

21. **Armitage GY, Berry WG (1987),** Statistical Methods. 7th Ed. Ames.

22. **Danzi S, Klein I. (2012):** Thyroid hormone and the cardiovascular system. Medical Clinics of North America. 96:257-268. doi: 10.1016/j. mcna.2012.01.006.

23. **Parkinson L, Cicerale S. (2016):** The Health Benefiting Mechanisms of Virgin Olive Oil Phenolic Compounds. Molecules.21(12):1734-1746. doi: 10.3390/molecules21121734.

24. **Fernandes RO, Dreher GJ, Schenkel PC, Fernandes TR, Ribeiro MF, *et al.* (2011):** Redox status and pro-survival/pro-apoptotic protein expression in the early cardiac hypertrophy induced by experimental hyperthyroidism. Cell Biochemistry and Function. 29(7):617-623. DOI: 10.1002/cbf.1796

25. **Vargas F, Rodríguez-Gómez I, Vargas-Tendero P, Jimenez E, Montiel M. (2012):** The renin–angiotensin system in thyroid disorders and its role in cardiovascular and renal manifestations. Journal of Endocrinology. 213:25–36. doi: 10.1530/JOE-11-0349.

26. **Takano AP, Diniz GP, Barreto-Chaves ML. (2013):** AMPK signaling pathway is rapidly activated by T3 and regulates the cardiomyocyte growth. Molecular and cellular Endocrinology. 376: 43–50. doi: 10.1016/j.mce.2013.05.024.

27. **Bhullar SK, Dhalla NS. (2022):** Angiotensin II-Induced Signal Transduction Mechanisms for Cardiac Hypertrophy. Cells. 11 (21): 3336. Doi.org/10.3390/cells11213336

28. **Vargas-Uricoechea H, Bonelo-Perdomo A, Sierra-Torres CH. (2014):** Effects of thyroid hormones on the heart. Clínica e Investigación en Arteriosclerosis. 26(6):296-309. Doi: 10.1016/j.arteri.2014.07.003.

29. **Kim BH, Cho KI, Kim SM, Kim J-Y, Choi BG, Kang JH, Jeon YK, Kim S-J, Kim YK, Kim IJ. (2012):** Irbesartan prevents myocardial remodeling in experimental thyrotoxic cardiomyopathy. Endocrinology journal. 59 (10):919-929. doi: 10.1507/endocrj.ej12-0111.

30. **Zeng SY, Yang L, Lu H-Q, Yan Q-J, Gao L, Qin X-P. (2019):** Rutaecarpine prevents hypertensive cardiac hypertrophy involving the inhibition of Nox4-ROS-ADAM17 pathway. Journal of Cellular and Molecular Medicine. 23(6):4196-4207. Doi.org/10.1111/jcmm.14308

31. **Martins D, Garcia LR, Queiroz DAR, Lazzarin T, Tonon CR, Balin PDS, Polegato BF, de Paiva SAR, Azevedo PS, Minicucci MF, Zornoff L. (2022):** Oxidative Stress as a Therapeutic Target of Cardiac Remodeling. Antioxidants (Basel).11(12):2371. doi.org/10.3390/antiox11122371

32. **Ruiz- Ortega M, Ruperez M, Lorenzo O, Esteban V, Blanco J, Mezzano S, Egido J. (2002):** Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. Kidney International. 62(Suppl 82): S12–S22. https://doi.org/10.1046/j.1523-1755.62.s82.4.x

33. **Campbell N.K., Fitzgerald H.K. and Dunne A. (2021):** Regulation of inflammation by the antioxidant haem oxygenase 1. Nature Reviews Immunology.21(7):411-425. DOI: 10.1038/s41577-020-00491-x

34. **Li F, Lu S, Zhu R, Zhou Z, Ma L, Cai L, Liu Z. (2011):** Heme oxygenase-1 is induced by thyroid hormone and involved in thyroid hormone preconditioning-induced protection against renal warm ischemia in rat. Mol. Cell. Endocrinology. 339(1-2):54-62. doi: 10.1016/j.mce. 2011.03.019.

35. **Ha YM, Ham SA, Kim YM, Lee YS, Kim HJ, Seo HG, Lee JH, Park MK, Chang KC. (2011):** β_1-adrenergic receptor-mediated HO-1 induction, via PI3K and p38 MAPK, by isoproterenol in RAW 264.7 cells leads to inhibition of HMGB1 release in LPS-activated RAW 264.7 cells and increases in survival rate of CLP-induced septic mice. Biochemical Pharmacology. 82:769-777. doi: 10.1016/j.bcp.2011.06.041.

36. **Araujo JA, Zhang M, Yin F. (2012):** Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. Front Pharmacol.;3:119. doi: 10.3389/fphar.2012.00119.

37. **Martín-Peláez S, Castañer O, Konstantinidou V, Subirana I, Muñoz-Aguayo D, Blanchart, G, Gaixas S, de la Torre R, Farré M, Sáez GT, Nyyssönen K, Zunft HJ, Covas MI, Fitó M. (2017):** Effect of olive oil phenolic compounds on the expression of blood pressure-related genes in healthy individuals. European journal of nutrition. 56, 663–670. doi.org/10.1007/s00394-015-1110-z

38. **Vazquez A, Sanchez-Rodriguez E, Vargas F, Montoro-Molina S, Romero M, Espejo-Calvo JA, Vilchez P, Jaramillo S, Olmo-García L, Carrasco-Pancorbo A, de la Torre R, Fito M, Covas MI, Martínez de Victoria E, Mesa MD. (2019):** Cardioprotective Effect of a Virgin Olive Oil Enriched with Bioactive Compounds in Spontaneously Hypertensive Rats. Nutrients.11(8):1728. doi: 10.3390/nu11081728.

39. **Massaro M, Scoditti E, Carluccio MA, Calabriso N, Santarpino G, Verri T, De Caterina R. (2020):** Effects of Olive Oil on Blood Pressure: Epidemiological, Clinical, and Mechanistic Evidence. Nutrients.12(6):1548. doi: 10.3390/nu12061548.

40. **Zhao X, Gu T. (2016):** Dysfunctional Hyperpolarization-Activated Cyclic Nucleotide-gated Ion Channels in Cardiac Diseases. Brazilian Journal of Cardiovascular surgery. 31(2):203-206. Doi. org/10.5935/1678-9741.20160030

41. **Almeida NA, Corderio A, Machado DS, Souza LL, Ortiga-Carvalho TM, Campos-de-Carvalho AC, Wondisford FE, Pazos-Moura CC. (2009):** Connexin 40 messenger ribonucleic activity is positively regulated by thyroid hormone (TH) acting in cardiac atria via the TH receptor. Endocrinology. 150: 546-554. doi: 10.1210/en.2008-0451.

42. **Wang J, Li ZX, Liu JH, Wang SP, Zhang M, Wang CH, Wang P, Zheng Z. (2018):** Different expression of connexin 43 between culprit arteries and non-culprit arteries and role of angiotensin II on expression of connexin 43 in non-culprit arteries. International Journal of Clinical and Experimental Pathology.11(1):382-390. PMID: 31938122; PMCID: PMC6957930.

43. **Ramachandran S, Xie LH, John SA, Subramaniam S, La lR. (2007):** A Novel Role for Connexin Hemichannel in Oxidative Stress and Smoking-Induced Cell Injury. PLoS One. 2(8): e712-724. doi. org/10.1371/journal.pone.0000712

44. **Klein I, Danzi S. (2016):** Thyroid Disease and the Heart. Current Problems in Cardiology. 41:65–92. doi. org/ 10.1161/ CIRCULATION AHA.106.678326

45. **Bhullar SK, Shah AK, Dhalla NS. (2021):** Role of angiotensin II in the development of subcellular remodeling in heart failure. Exploration of Medicine. 2:352–371. doi.org/10.37349/emed.2021.00054

46. **Zhou Y. Jing S, Liu S, Shen X, Cai L, Zhu C, Zhao Y, Pang M. (2022):** Double-activation of mitochondrial permeability transition pore opening via calcium overload and reactive oxygen species for cancer therapy. J Nanobiotechnology.20(1):188. doi: 10.1186/s12951-022-01392-y.

47. **Raguthu CC, Gajjela H, Kela I, Kakarala CL, Hassan M, Belavadi R, Gudigopuram SVR, Sange I. (2022):** Cardiovascular Involvement in Thyrotoxicosis Resulting in Heart Failure: The Risk Factors and Hemodynamic Implications. Cureus. 13;14(1): e21213. doi: 10.7759/cureus.21213.

48. **Al-Shudiefat AA, Ludke A, Malik A, Jassal DS, Bagchi AK, Singal PK. (2022):** Olive oil protects against progression of heart failure by inhibiting remodeling of heart subsequent to myocardial infarction in rats. Physiological Reports. 10(15): e15379. doi: 10.14814/phy2.15379.

49. **Lu Y, Zhao J, Xin Q, Xin Q, Yuan R, Miao Y, Yang M, Mo H, Chen K, Cong W. (2014):** Protective effects of oleic acid and polyphenols in extra virgin olive oil on cardiovascular diseases. Food Sci. Hum. Wellness.13(2):529-540. Doi.org/10.26599/ FSHW.2022. 9250047

50. **Ago T, Sadoshima J. (2015):** From contractile enhancement to pathological hypertrophy: Angiotensin II–induced Nox2-mediated reactive oxygen species. Journal of the American College of Cardiology.66(3):273–277. doi: 10.1089/ ars.2012.4641.

51. **Klein I, Danzi S. (2007):** Thyroid disease and the heart. Circulation. 116:1725-1735. doi: 10.1161/ CIRCULATION AHA.106.678326.

52. **Di Nicolantonio JJ, O'Keefe JH. (2017):** Good fats versus bad fats: a comparison of fatty acids in the promotion of insulin resistance, inflammation, and obesity. Molecular Medicine. 114: 303-307. PMID: 30228616; PMCID: PMC6140086.

زيت الزيتون البكر الممتاز يحمي من الخلل الوظيفي لعضلة القلب الناتج عن فرط نشاط الغدة الدرقية المستحث في ذكور الجرذان من خلال تقليل التعبير عن مستقبلات الانجيوتنسين-II

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خلفية وهدف البحث: ينتشر الاختلال الوظيفي لعضلة القلب المصاحب لفرط نشاط الغدة الدرقية ويرتبط بالإجهاد التأكسدي ونظام الرينين أنجيوتنسين. وتهدف هذه الدراسة إلى تقييم التأثيرات الوقائية لزيت الزيتون البكر الممتاز على عضلة القلب في هذه الحالات عن طريق مستقبلات الأنجيوتنسينII وإنزيم (HO-١) المضاد للأكسدة.

مواد وطرق البحث: تم تقسيم ٣٠ من ذكور الجرذان البالغة إلى الجرذان الضابطة، الجرذان المصابة بفرط هرمون الغدة الدرقية المستحث (الثيروكسين) (١٠٠ميكروجم/كجم/IP) مرة واحده يوميا /٦ ايام اسبوعيا" / ٤ أسابيع، الجرذان المصابة بفرط هرمون الغدة الدرقية المستحث المعالجة بزيت الزيتون البكر بداية من الاسبوع الثالث من التجربة (١مليتر/١٠٠كجم) مره واحدة يوميا/٦ ايام اسبوعيا" وحتى انتهاء التجربة.

نتائج البحث: أظهرت النتائج ان زيت الزيتون الصافي ادى الى انخفاضًا في أوزان الجرذان والوزن المطلق للبطين الايسر مع زيادة ضربات القلب وتحسن في رد الفعل لعقار الايزو وزيادة القوة المتولدة نتيجة الانقباض مقارنة بوزن البطين الايسر ومعدل تولد القوة مقابل الزمن مع ارتفاع معدل سريان المحلول المغذي للقلب بالإضافة لانخفاض في حالة الاكسدة وفي انزيم HO-I ومستوى التعبير عن مستقبلات الانجيوتينسن -II مع ظهور خلايا عضلية منظمة ومتفرعة بعكس التضخم في المجموعات المصابة.

الإستنتاج: زيت الزيتون الصافي كان قادرًا على توفير حماية جزئية من تضخم و خلل وظائف القلب في حالات فرط نشاط الغدة الدرقية عن طريق تخفيف الحالة المؤكسدة ومستوى التعبير عن مستقبلات الانجيوتينسن -II