THE EFFECT OF COENZYME Q10 AND/OR Silymarin ON RENALASE GENE EXPRESSION OF CARDIORENAL SYNDROME IN ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: Therapeutic strategies in cardiorenal syndrome (CRS) should be directed to the cardio-renal connectors like renalase. Coenzyme Q10 (CoQ10) and silymarin are natural anti-oxidant and anti-inflammatory agents.

Objectives: Assessing the effect of CoQ10 and/or Silymarin on renalase gene expression in CRS induced by high fructose diet (HFD) in male rats.

Patients and Methods: Fifty adult male albino rats of local strain were divided into 5 equal groups and subjected to the following regimens for 8 weeks: Group I: Supplemented orally with 1 ml of 2% aqueous solution of tween 80. Group II: Received HFD in the form of 30% fructose in drinking water. Group III: Received HFD and CoQ10 orally at a dose of 20 mg/kg/day dissolved in 2% tween-80 aqueous solution. Group IV: Received HFD and Silymarin orally at a dose of 200 mg/kg/day dissolved in 2% tween-80 aqueous solution. Group V: Received HFD and both CoQ10 and silymarin in the same regimen as described before.

Results: CoQ10 and/or Silymarin significantly decreased plasma lipid profile, cardiac troponin-I, creatinine, malondialdehyde (MDA) & tumor necrosis factor alpha (TNF-α) levels when compared to HFD group. On the other hand, CoQ10 and/or silymarin caused significant increase in plasma catalase level & renalase gene expression in kidney tissue when compared to HFD group. They also improved HFD -induced cardiac and renal fibrosis.

Conclusion: CoQ10 and Silymarin induced improvement in HFD-induced CRS as they possessed cardio-protective, reno-protective, hypo-lipidimic, anti-oxidant, anti-inflammatory and anti-fibrotic activities, as well as increased renalase expression.

Key words: Coenzyme Q10; Silymarin; Renalase; Cardiorenal Syndrome; High fructose diet.

INTRODUCTION

The maintenance of cardiovascular and renal hemostasis is dependent upon fine interactions between the heart and kidney (Hadjiphilippou and Kon, 2015). Cardiorenal syndrome (CRS) is a clinico-pathologic disorder in which a primary insult in the kidney or in the heart initiates a series of secondary functional and morphologic responses in the other organ (Athwani et al., 2017). Several mechanisms are involved in the pathophysiology of CRS such as
hemodynamic mechanism, neurohormonal adaptations, oxidative stress and inflammation, endothelial dysfunction and atherosclerosis (Naranjo et al., 2017).

High fructose diet (HFD) induces CRS (Jia et al., 2014). It can lead to some problems as risk factors for kidney and cardiac dysfunction such as obesity, hyperuricemia, dyslipidemia, hypertension, diabetes mellitus, and induces inflammation and oxidative stress (Yerlikaya et al., 2017).

Coenzyme Q10 (CoQ10) is a vitamin-like substance presents in most eukaryotic cells especially in the mitochondria. CoQ10 is essential for energy production in electron transport chain (Pahari et al., 2016). Also, CoQ10 is considered the only lipid-soluble anti-oxidant produced by humans. It acts as an anti-inflammatory agent (Farsi et al., 2017). It is a potent gene regulator and improves human immunity. CoQ10 supplementation can be used due to its neuro-protective, anti-carcinogenic, anti-diabetic, and hepato-protective effects (Garrido-Maraver et al., 2014).

Silymarin is a natural polyphenolic flavonoid isolated from Silybum marianum. Silymarin has anti-oxidant, anti-inflammatory and anti-apoptosis properties (Razavi and Karimi, 2016). It also has immune-modulatory and anti-fibrotic activities. Silymarin can be used as hepato-protective, neuro-protective and anti-diabetic agent (Darvishi-Khezri et al., 2017).

Renalase is a new renal hormone with mono-amine oxidase (MAO) activity (Dziedzic et al., 2017). Kidney is the main organ of renalase expression and secretion, but its expression also takes place in other tissues such as heart, vascular endothelium, liver and nervous system (Skrzypczyk et al., 2017). Renalase can metabolize circulating catecholamines and act as a cytokine to regulate cell functions (Wu et al., 2018). Decreased renalase level leads to deleterious long-term effects on heart and kidney which finally causes CRS (Wybraniec and Mizia-Stec, 2016).

The aim of the present study was to clarify the effect of CoQ10 and/or silymarin on renalase gene expression in CRS induced by HFD in male rats.

**PATIENTS AND METHODS**

**Animals:**

The present study was conducted on fifty adult male rats of local strain (weighing 120 – 160 grams). Rats were kept in suitable stainless steel cages (50×50×65 cm in size, 5 rats per cage). Rats were kept for one week under prevailing atmospheric conditions before the start of the experiment to ensure laboratory acclimatization.

Rats were housed under appropriate conditions of controlled humidity. They were maintained at constant room temperature and suitable illumination conditions (normal light/dark cycle). Rats were allowed to ordinary rat chow and fresh tap water ad-libitum. The present study was carried out in the animal house of Physiology Department, Faculty of Medicine (Girls), Al-Azhar University.

**Drugs:**

Fructose was purchased as powder from Specialized Food Industry Company (King M); Badr City, Egypt. Tween 80 was purchased as solution from ADWIC
Company; Cairo, Egypt. It can be used as a vehicle to emulsify hydrophobic substances (Freitag et al., 2015). CoQ10 was purchased as capsules from MEPACO Company; Sharkeya, Egypt. Silymarin (Legalon) was purchased as capsules from CID Company; Giza, Egypt.

**Experimental Design:**

The rats were divided into 5 equal groups and subjected to the following regimens for 8 weeks (6 consecutive days/week): **Group I** (control group): Rats fed on ordinary rat chow with free access to tap water. Each rat was supplemented orally with 1 ml of 2% aqueous solution of tween 80/day. **Group II** (HFD group): Rats received HFD in the form of 30% fructose in drinking water (Cho et al., 2017). **Group III** (CoQ10 group): Rats received HFD and CoQ10 orally at a dose of 20 mg/kg/day dissolved in 2% tween-80 aqueous solution (Singh, 2015). **Group IV** (Silymarin group): Rats received HFD and Silymarin orally at a dose of 200 mg/kg/day dissolved in 2% tween-80 aqueous solution (Jamila et al., 2017). **Group V** (CoQ10 & Silymarin group): Rats received HFD with CoQ10 and Silymarin in the same regimen as described before.

The body weight of the rats were measured and recorded weekly for all groups. At the end of the experimental period, rats were fasted for 12 hours, and then blood samples were collected under light ether anesthesia from retro-orbital sinuses by capillary tubes (Simmons and Brick, 1970). The spurring blood was collected in ethylene diamine tetra-acetic acid (EDTA) coated tubes and centrifuged at 3500 rpm for 15 min for plasma collection. Samples were stored frozen at -80°C until biochemical analysis.

**Biochemical analysis:**

Plasma high density lipoprotein cholesterol (HDL-C), triglycerides and total cholesterol levels were measured by quantitative-enzymatic-colorimetric procedure (França et al., 2018). They measured by using colorimetric assay kits from Cayman Chemical Company, Calbiochem Company and BioMed diagnostic Company respectively. Plasma low density lipoprotein cholesterol (LDL-C) was calculated from the values of total cholesterol, HDL-c and triglycerides using Friedewald equation: LDL-C (mg/dl) = (total cholesterol) – (HDL-C) – (triglycerides/5) (Friedewald et al., 1972). Plasma cardiac troponin I level was measured by quantitative determination using a rat cardiac troponin-I enzyme-linked immunosorbent assay (ELISA) kit from Kamiya Biomedical Company (Collinson et al., 2001). Plasma creatinine level was determined using a creatinine kit from BioMed diagnostic Company (Schirmeister et al., 1964). Plasma MDA and catalase levels were detected by colorimetric method using kits from Bio-diagnostic Company (Aebi, 1984). Plasma TNF-α level was measured by quantitative measurement using a rat TNF-α ELISA kit from Ray-Biotech Company (Engelmann et al., 1990).

**Polymerase chain reaction (PCR) technique:**

For detection of renalase gene expression in kidney tissue, RNA was extracted, reversely transcribed into cDNA and amplified by PCR, and then detected using agarose gel electrophoresis (Bustin et al., 2009).
Histo-pathological examination:

Heart and kidney tissue specimens were collected for histological examination by the light microscope. Specimens were fixed in 10% neutral buffered formalin and processed for paraffin thin sections (Bancroft and Stevens, 1996). Sections were stained with Masson’s trichrome for detection of connective tissue deposits and fibrosis (Hu et al., 2019).

Statistical Analysis:

Statistical analysis was done by using statistic package for social science version 20 (SPSS, 20) for windows. Statistical analysis of variance between mean values of different groups was performed using one-way analysis of variance (ANOVA) followed by Bonferroni Post Hoc test. Quantitative data were expressed by mean ± standard deviation (S.D.). The values of P< 0.05 were considered statistically significant.

RESULTS

I- HFD caused significant increase in plasma lipid profile, cardiac troponin-I, creatinine, MDA and TNF-α levels, and significant decrease in plasma catalase level and renalase gene expression in kidney tissue when compared to control group. HFD caused insignificant changes in body, heart and kidney weights when compared to control group.

II- Administration of CoQ10 and/or Silymarin caused significant decrease in plasma lipid profile, cardiac troponin-I, creatinine, MDA and TNF-α levels, and significant increase in plasma catalase level and renalase gene expression in kidney tissue when compared to HFD group. CoQ10 and/or Silymarin caused insignificant changes in body, heart and kidney weights when compared to HFD group.

III- Co-administration of CoQ10 and Silymarin caused insignificant changes in body, heart and kidney weights, plasma triglycerides, total cholesterol, LDL-C, cardiac troponin-I, creatinine, MDA, catalase and TNF-α levels, while it caused significant increase in plasma HDL-C level and renalase gene expression when compared to CoQ10 group.

IV- Co-administration of CoQ10 and Silymarin caused insignificant changes in body, heart and kidney weights, plasma total cholesterol, LDL-C, cardiac troponin-I, creatinine, MDA and catalase levels, while it caused significant decrease in plasma triglycerides and TNF-α levels, and caused significant increase in plasma HDL-C level and renalase gene expression when compared to Silymarin group.
### Table (1): Effect of HFD, CoQ10 and/or Silymarin on different parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control group)</th>
<th>Group II (HFD group)</th>
<th>Group III (CoQ10 group)</th>
<th>Group IV (Silymarin group)</th>
<th>Group V (CoQ10 &amp; Silymarin group)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>176.5 ± 9.14</td>
<td>177 ± 20.57</td>
<td>173.5 ± 21.08</td>
<td>171.5 ± 13.75</td>
<td>169 ± 22.82</td>
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<tr>
<td><strong>Heart weight (g)</strong></td>
<td>0.557 ± 0.04</td>
<td>0.562 ± 0.09</td>
<td>0.566 ± 0.07</td>
<td>0.551 ± 0.04</td>
<td>0.556 ± 0.04</td>
</tr>
<tr>
<td><strong>Kidney weight (g)</strong></td>
<td>1.102 ± 0.21</td>
<td>1.169 ± 0.22</td>
<td>1.112 ± 0.21</td>
<td>1.136 ± 0.2</td>
<td>1.1 ± 0.15</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td>61.8 ± 9.8</td>
<td>109.7 ± 14.5</td>
<td>82.7 ± 8.69</td>
<td>88.8 ± 8.71</td>
<td>69.4 ± 10.77</td>
</tr>
<tr>
<td><strong>Total cholesterol (mg/dl)</strong></td>
<td>140.6 ± 15.07</td>
<td>235.5 ± 36.83</td>
<td>180.4 ± 21.53</td>
<td>174.4 ± 18.88</td>
<td>166.9 ± 17.45</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dl)</strong></td>
<td>58.6 ± 3.56</td>
<td>25.2 ± 5.39</td>
<td>36.4 ± 7.04</td>
<td>40 ± 4.98</td>
<td>49.6 ± 8.59</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dl)</strong></td>
<td>69.64 ± 17</td>
<td>188.36 ± 39.36</td>
<td>127.46 ± 26</td>
<td>116.64 ± 19.17</td>
<td>103.42 ± 18.41</td>
</tr>
<tr>
<td><strong>Cardiac troponin I (ng/ml)</strong></td>
<td>0.019 ± 0.01</td>
<td>0.088 ± 0.02</td>
<td>0.037 ± 0.01</td>
<td>0.037 ± 0.01</td>
<td>0.029 ± 0.01</td>
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<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td>0.1650 ± 0.05</td>
<td>1.6390 ± 0.71</td>
<td>0.5790 ± 0.22</td>
<td>0.5240 ± 0.18</td>
<td>0.3070 ± 0.11</td>
</tr>
<tr>
<td><strong>MDA (nmol/ml)</strong></td>
<td>5.539 ± 1.71</td>
<td>80.4 ± 22.81</td>
<td>27.7 ± 9.85</td>
<td>35.46 ± 15.3</td>
<td>24.51 ± 6.93</td>
</tr>
<tr>
<td><strong>Catalase (U/ml)</strong></td>
<td>192.78 ± 19.44</td>
<td>86.04 ± 17.4</td>
<td>173.04 ± 15.87</td>
<td>173.87 ± 15.42</td>
<td>188.75 ± 15.56</td>
</tr>
<tr>
<td><strong>TNF-α (pg/ml)</strong></td>
<td>31.84 ± 4.22</td>
<td>116.61 ± 14.24</td>
<td>67.49 ± 12.43</td>
<td>78.29 ± 11.74</td>
<td>60.4 ± 12.19</td>
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<tr>
<td><strong>Renalase gene expression</strong></td>
<td>1.026 ± 0.06</td>
<td>0.292 ± 0.12</td>
<td>0.674 ± 0.1</td>
<td>0.660 ± 0.1</td>
<td>0.843 ± 0.09</td>
</tr>
</tbody>
</table>

a = Significant values versus group I (control)  
b = Significant values versus group II (HFD)  
c = Significant values versus group III (CoQ10)  
d = Significant values versus group IV (Silymarin)
Histo-pathological results:

Figure (1): Microscopic examination of cardiac tissues: (A) Control group showing normal density of very thin collagen fibers in-between the cardiac muscle fibers, (B) HFD group showing marked fibrosis in-between the disorganized cardiac muscle fibers (arrows), while there were marked improvement in (C) CoQ10 group, (D) Silymarin group, and (E) CoQ10 & Silymarin group (Masson's trichrome-X 100).

Figure (2): Microscopic examination of kidney tissues: (A) Control group showing normal density of very thin collagen fibers in the tubular wall, (B) HFD group showing marked fibrosis in the tubule-interstitial tissues (arrows) besides shrunken renal corpuscles (arrow head), while there were marked improvement in (C) CoQ10 group, (D) Silymarin group, and (E) CoQ10 & Silymarin group (Masson's trichrome-X 100).
DISCUSSION

In the present study, HFD caused insignificant changes in body, heart and kidney weights when compared to control group. This agreed with Chou et al. (2018) and Jensen et al. (2018) results. HFD induced dyslipidemia. (Chen et al., 2017) stated that HFD augmenting lipogenesis through up-regulating liver X receptor -α (LXR -α). HFD caused increase of cardiac troponin-I level. (Park et al., 2018) mentioned that HFD induced this result by inactivation of 5’ adenosine monophosphate-activated protein kinase (AMPK) signaling pathway.

HFD caused increase of creatinine level. Yang et al. (2015) explained that HFD can increase renal expression of renal urate transporter 1 (URAT1) and causes hyper-uricemia which induces inflammation and subsequently kidney injury and dysfunction.

HFD induced oxidative stress in this work agreed with Kosuru et al. (2018) who reported that HFD induces peroxidation that results in increased MDA level. HFD induced inflammation which agreed with Jensen et al. (2018) and Suriano et al. (2018) who reported that HFD increases intestinal bacterial overgrowth and permeability. So, it can facilitate translocation of bacterial endotoxins to the liver which stimulates Kupffer cells leading to inflammatory response and cytokines production.

HFD in the present work caused significant decrease in renalase gene expression in kidney tissue when compared to control group. To our knowledge, this was the first study to detect the effect of HFD on renalase gene expression which may be explained by the fibrosis observed in the kidney tissues of HFD group. Wu et al. (2017) reported that the expression level significantly diminishes in sub-totally nephrectomized rats due to the progressive renal fibrosis. Xie (2017) reported that HFD induced fibrosis through stimulation of alpha smooth muscle-actin (α–SMA).

In the present study, CoQ10 caused insignificant changes in body, heart and kidney weights when compared to HFD group which agreed with Garjani et al. (2011) and Barden et al. (2018). CoQ10 caused hypolipidemic effect which agreed with Rahmani et al. (2018) who reported that CoQ10 induces gene expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) that inhibits lipogenesis. CoQ10 showed decrease in creatinine and cardiac troponin-I levels which agreed with Fatima et al. (2015) and Tachampa et al.(2018) who attributed these effects to the anti-oxidant and anti-inflammatory properties of CoQ10.

CoQ10 in this work showed anti-oxidant activity. Hormozi et al. (2018) attributed this effect to the ability of CoQ10 to regenerate endogenous antioxidants such as vitamins C and E. Administration of CoQ10 showed anti-inflammatory activity. Rahmani et al. (2018) attributed this effect to the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway by CoQ10.

CoQ10 caused significant increase in renalase gene expression in kidney tissue when compared to HFD group. To our knowledge, this is the first study to detect the effect of CoQ10 on renalase gene expression. CoQ10 showed anti-fibrotic
activity. (Chen et al., 2018) attributed this effect to the inhibition of transforming growth factor beta 1 (TGF-β1) expression by CoQ10.

In the present study, Silymarin caused insignificant changes in body, heart and kidney weights when compared to HFD group which agreed with Wang et al. (2018) results. Silymarin caused hypo-lipidimic effect. Sharma et al. (2018) attributed this effect to the suppression of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity by Silymarin.

Silymarin showed decrease in cardiac troponin-I and creatinine levels. Avci et al. (2017) and Ustyol et al. (2017) attributed these effects to the anti-oxidant and anti-apoptotic properties of silymarin.

Silymarin showed anti-oxidant activity by decreasing MDA level and increasing catalase level. Vivekanandan et al. (2018) attributed this effect to the ability of Silymarin to restore non-enzymatic antioxidant levels such as reduced glutathione, vitamins C, A and E. Kwon et al. (2013) stated that Silymarin enhances hepatic reduced glutathione generation by elevating cysteine availability and inducing cystine synthesis.

Silymarin showed anti-inflammatory activity. Zhang et al. (2013) attributed this effect to the inhibition of NF-κB pathway by silymarin. Silymarin caused significant increase in renalase gene expression in kidney tissue when compared to HFD group. To our knowledge, this is the first study to detect the effect of silymarin on renalase gene expression. Silymarin showed anti-fibrotic activity. Meng et al. (2019) attributed this effect to the inhibition of TGF-β1 signaling pathway by silymarin. Combined CoQ10 & Silymarin showed improvement of all studied parameters except insignificant change in body, heart and kidney weights. There were no previous studies discussed the effect of both. Therefore, the obtained results reflected the cumulative action of both with their underlying mechanisms of action which discussed before.

CONCLUSION
Administration of HFD induced dyslipidemia, heart and kidney injury, oxidative stress, inflammation, fibrosis and significant decrease in renalase gene expression in kidney tissue, while administration of CoQ10 and/or Silymarin induced hypo-lipidimic, cardio-protective, reno-protective, anti-oxidant, anti-inflammatory and anti-fibrotic activities, and significant increase in renalase gene expression in kidney tissue. This suggested that CoQ10 and Silymarin provided a protective option for combating CRS through several mechanisms.

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Tأثير الإنزيم المساعد كيو ١٠ والسليمارين على التعبير الجيني للريناالاز لمتلازمة القلب والكلى في ذكور الجرذان البالغة

جيهان أحمد يوسف- منى جمال الدين الغانم- غادة محمد صلاح الدين- سارة نجدي محمود

قسم الفسيولوجي- كلية الطب (بنات)- جامعة الأزهر

خلفية البحث: من الأفضل أن توجه الاستراتيجيات العلاجية في متلازمة القلب والكلى إلى الروابط بينهما مثل الريناالاز. الإنزيم المساعد كيو ١٠ والسليمارين من المواد الطبيعية المضادة للأكسدة والالتهابات، كما أن لهما دور وقائي للقلب والكلى.

الهدف من البحث: تهدف هذه الدراسة إلى توضيح تأثير الإنزيم المساعد كيو ١٠ أو السليمارين أو كليهما معا على التعبير الجيني للريناالاز في متلازمة القلب والكلى الناتجة عن التغذية عاليا الفركتوز في ذكور الجرذان البالغة.

المرضي و طرق البحث: تم إجراء الدراسة الحالية لمدة ٨ أسابيع على ٥٠ من ذكور الجرذان البالغة. وتم تقسيمهم إلى ٥ مجموعات متساوية:

المجموعة الأولى : (مجموعة ضابطة) تناولت غذاء الجرذان المعتاد وتم إعطاؤها كل جرذ ١ مللي من محلول مائي من التوبيس ٨٠ تركيز ٢٪ عن طريق الفم.

المجموعة الثانية : تناولت تغذية عاليا الفركتوز بنسبة ٣٢٪ مذاب في الماء.

المجموعة الثالثة : تناولت تغذية عالية الفركتوز بالإضافة إلى الإنزيم المساعد كيو ١٠ بجرعة ٢٠ جم/كم/اليوم عن طريق الفم.

المجموعة الرابعة: تناولت تغذية عالية الفركتوز بالإضافة إلى السليمارين بجرعة ٢٠٠ جم/كم/اليوم عن طريق الفم.

المجموعة الخامسة: تناولت تغذية عالية الفركتوز بالإضافة إلى الإنزيم المساعد كيو ١٠ والسليمارين، وتم إعطاؤهم نفس الجرعات كما ذكر في المجموعتين السابقتين.
وفي نهاية التجربة تم تجميع عينات الدم وأنسجة الكلى وتم قياس مستويات دهون الدم وتربوئين القلب-I و الكرياتينين وعامل الإجهاد التاكسدي والكالاتز وعامل نخرالورم ألفا في البلازما، والتعبير الجيني للرينالاز في الكلى، كما تم فحص التليف في نسيج القلب والكلي.

النتائج: نتج عن إعطاء الإنزيم المساعد كيو 10 أو السليمارين أو كليهما مع انخفاض ذو دلالات إحصائية في مستويات دهون الدم وتربوئين القلب-I و الكرياتينين وعامل الإجهاد التاكسدي وعامل نخرالورم ألفا في البلازما مقارنة بالمجموعة التي تتغذى على الفركتوز، بينما ارتفع مستوى الكالاتز في البلازما والتعبير الجيني للرينالاز في الكلى مقارنة بالمجموعة التي تتغذى على الفركتوز ارتفاع ذو دلالة إحصائية. كما حدث تحسن في تليف القلب والكلي.

الاستنتاج: نتج عن إعطاء الإنزيم المساعد كيو 10 والسليمارين حماية القلب والكلي وتقليل دهون الدم والأجهد التاكسدي والإنجابات وتليف القلب والكلى وزيادة كبيرة في مضادات الإجهاد التاكسدي والتعبير الجيني للرينالاز في أنسجة الكلى.