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Targeting Asprosin/ Nrf2 Pathway in the Potential Protective Effect of Co-enzyme Q10 and/or Exercise on High-Fat Diet Induced Non-alcoholic Fatty Liver Disease in Rats

Reham M. Ibrahim[1*], *Hadeer Abd El-shafy Fouad*[2], *Noha Osama El-Shaer*[1], *Noha Ibrahim Hussien*[1], *Marwa H. Muhammad* [1]

[1]Department of Physiology, Faculty of Medicine, Benha University, Qalubiya, Egypt.
[2]Department of physiology, Faculty of medicine, Suez University, Suez, Egypt.

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- Nonalcoholic fatty liver disease
- Co-enzyme Q10
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- Nrf2.

Abstract

Background: The most prevalent liver disease which is rising globally is Non-alcoholic Fatty Liver Disease (NAFLD). Asprosin a newly discovered glucogenic hepatic targeting adipokine has gained great interest. Coenzyme Q 10 (CoQ10) as antioxidant dietary supplement and exercise as an optimal non pharmacological strategy were chosen. **Aim:** To assess hepato-protective effect of CoQ10 and/or exercise on NAFLD rat model and exploring potential involvement of asprosin/ nuclear factor erythroid 2–related factor 2 (Nrf2) in such effect. **Materials and Method:** Five groups of rats were conducted for 8 weeks experimental period. Control rats were fed balanced diet. High-fat diet (HFD) group was fed HFD. HFD + exercise group, rats were fed HFD and performed swimming exercise for 60 min/5 days/week. HFD + CoQ10 group, HFD fed rats received CoQ10 20 mg/kg/day/ orally. HFD + CoQ10+ exercise group, rats were fed HFD and performed swimming exercise along with receiving CoQ10. **Results:** Our findings revealed that HFD led to NAFLD characteristics at both biochemical and histopathological levels. Evident dyslipidemia, insulin resistance, and oxidative stress were documented. These findings were improved by CoQ10 and/or exercise. Asprosin-decline and Nrf2 augmentation were among contributing mechanisms. **Conclusion:** CoQ10 and/or exercise had a protective effect on a rat model of NAFLD. Asprosin lowering expressions while, enhancing Nrf2 protein expression were among underlying protective impact.

Corresponding author: Reham Mohammed Ibrahim , Physiology Department, Faculty of Medicine, Benha University, Qalubiya, Egypt. E. Mail: reham.mohamed@fmed.bu.edu.eg. Mobile: 002 01222349445

Introduction

Obesity and its related comorbidities have gained international attention in recent years **[1].** Non-alcoholic fatty liver disease (NAFLD) exhibits a strong correlation with obesity, and its incidence is perpetually increasing across globe. Approximately one-quarter of global population is impacted by this condition **[2].** NAFLD represents most widespread form of diffuse liver pathology, characterized by accumulation of lipid deposits within hepatic cells. This disorder is a major factor in liver-associated morbidity and mortality, with a propensity to evolve into advanced stages, including cirrhosis, worsening fibrosis, and non-alcoholic steatohepatitis (NASH), ultimately leading to critical conditions like carcinoma **[3, 4]**. In addition, NAFLD is a critical precipitating factor for several diseases, as type-2 diabetes and cardiovascular diseases **[5].**

Asprosin has been recently identified as an adipokine hormone released from white adipose tissue **[6].** It is a liver-targeting glucose-regulating peptide hormone **[7].** Excess asprosin level was detected in obesity and insulin resistance **[6, 8].** Thus, it could be a cross-talk between adipose tissue and liver. Moreover, numerous studies have revealed a robust association between asprosin and development of obesity, as well as its accompanying cardiovascular complications. Additionally, immune and inflammatory disorders have also been implicated **[9, 10].** However, its implication in pathogenesis and protection against NAFLD is still unclear.

NAFLD is defined by an abnormal buildup of fat in the liver. It arises due to an imbalance between the influx and production of lipids in the liver, and their breakdown through β-oxidation and subsequent export **[11].** Subsequent lipotoxicity generates a series of reactive oxygen species (ROS) resulting in an oxidative stress state with lipid peroxidation, protein and DNA damage. In addition, it causes disruption in several metabolic pathways and demonstrating insulin resistance. Consequently, oxidative stress driven by lipogenesis is regarded as predominant factor contributing to hepatic injury and progression of disease in context of NAFLD **[12].**

An essential natural transcription factor that can protect cells from oxidative stress is nuclear factor erythroid-2-related factor 2 (Nrf2) **[13].** Heme-oxygenase-1 is one of cyto-defenses that are promoted to transcript by Nrf2's binding of antioxidant response element inside nucleus **[14].** The probable significance of Nrf2 in NAFLD and potential ameliorating effects of Nrf2 activators have garnered significant attention, as oxidative stress is a fundamental aspect of NAFLD pathogenesis.

Coenzyme Q10 (CoQ10), is a naturally occurring antioxidant. It is found in plenty of animal and plant foods. Beef liver and heart and chicken are highest in CoQ10 content. Corn oil, olive oil, and peanut oil are best CoQ10 plant-based food **[15].** It is predominantly situated within inner mitochondrial membrane, playing a crucial role in ATP synthesis and facilitating electron transport processes **[16].** It functions a free radical quencher **[17].** In addition, it is a well-known anti-adipogenic factor **[18].** This shed light on reasonability of usage CoQ10 to counteract lipogenic-induced oxidative stress in NAFLD.

In general, excessive calorie consumption and a sedentary lifestyle contribute to the progression of NAFLD. As clinical evidence suggests, lifestyle changes can serve as a primary approach to managing NAFLD **[19].** Furthermore, exercise can maintain metabolic health through modulation of lipogenesis, mitochondrial function, and oxidative homeostasis **[20]**. Therefore, exercise is a potent non-pharmacological intervention for addressing NAFLD.

Given this context, the aim of the current study was to assess the potential protective effects of CoQ10 and/or exercise on NAFLD in rats induced by a high-fat diet. Additionally, the study sought to elucidate the role of the asprosin/Nrf2 pathway as a possible underlying mechanism.

2. Material and methods

2.1 Experimental animals

Thirty adult male Wistar rats (weight 130–150g) were obtained from Animal House of Faculty of veterinarian medicine, Benha University, Egypt. Rats were maintained on standard pellet diet and water ad libitum. These rats were housed in metallic cages at a temperature of 25°C. A one-week acclimatization phase was provided for rats to adapt to environmental conditions prior to commencing experiment. All techniques and protocol of this study were revised and authorized by Ethical Committee of Care and Use of Experimental animals, Faculty of Medicine, Benha University, Egypt (Approval No. MD 9-6-2023).

2.2 Experimental Design

The rats were randomly assigned to five distinct groups (n = 6). The **Control group** received a standard balanced diet consisting of 11.4% fat, 25.8% protein, and 62.8% carbohydrates. The **HFD group** was fed a high-fat diet (HFD) (34% fat, 19% protein, and 47% carbohydrates) for 8 weeks to induce NAFLD **[21]**. In the **HFD + exercise group**, rats were fed the HFD and participated in a swimming exercise regimen for 60 minutes, 5 days per week, over the 8-week period **[22].** The **HFD + CoQ10 group** consisted of HFD-fed rats that were also given CoQ10 orally at a dose of 20 mg/kg/day for 8 weeks **[23].** The CoQ10 was sourced from MEPACO Company, Heliopolis, Egypt. Lastly, in the **HFD + CoQ10 + exercise group**, the rats were fed the HFD, performed swimming exercises for 60 minutes, 5 days per week, and were administered CoQ10 orally at 20 mg/kg/day for 8 weeks.

The **Swimming exercise training protocol was as follow:** rats were adapted to swimming in a cylindrical tank of diameter 80 cm and depth 90 cm. It was filled with 60 cm water at temperature of 33 - 36°C. For two days, rats were permitted to swim for fifteen minutes for 2 days then; duration gradually increased until 60 min, on 5th day of first week of training then continued till end of 8th weeks at a rate of 5 days/week **[22].**

At end of experimental period, overnight-fasting animals were anesthetized with 3% pentobarbital (0.15 ml/100 g bw, i.p.) **[24]**

To isolate serum for biochemical analysis of asprosin, lipid profile, glucose, insulin, and aminotransferases, Blood specimens were procured from retro-orbital venous plexus and immediately processed via centrifugation at a speed of 3000 rpm for a period of 10 minutes. Liver tissue was ultimately collected and separated into two parts. One part was promptly frozen in liquid nitrogen and stored at -80°C for further evaluation, including hepatic triglyceride content,

oxidative stress parameters, and Nrf2 protein levels, which were analyzed through western blotting and Oil Red O staining techniques. The other part was put in 10% buffered neutral formalin for histopathological evaluation.

2.3 Measurements of serum lipid profile and asprosin levels

Serum levels of Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined biochemically using diagnostic kits provided by Biomed Diagnostics, Egypt.

The asprosin levels were quantified using Rat (Asprosin) ELISA Kit (REF: DZE21112025, LOT: 202206), which was sourced from Shanghai Sunred Biological Technology Co., located in Shanghai, China.

2.4 Assessment of serum glucose, insulin and their-related parameters

Serum glucose levels were measured using Biomed Diagnostics (Egypt) kits. Elabscience (China) ELISA reagent was employed to measure serum insulin. To assess insulin resistance and sensitivity conditions, following parameters were tested.

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was calculated using the following formula: HOMA-IR = fasting insulin (μU/L) × fasting glucose (mmol/L) / 22.5 **[25].** Additionally, the Quantitative Insulin-Sensitivity Check Index (QUICKI) was determined using this equation: QUICKI = 1 / [log fasting insulin (μU/ml) + log fasting glucose (mg/dl)] **[26].**

2.5 Assessment of hepatic TGs and oxidative stress-related parameters

Approximately 100 mg of frozen tissue samples were finely chopped and homogenized on ice in 1 mL of cold phosphate-buffered saline (PBS; pH 7.4, 0.1 M). The resulting homogenates were then centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was carefully collected for the assessment of oxidative stress markers and hepatic triglyceride levels.

Lipid peroxidation was determined by measuring malondialdehyde (MDA) levels using the MDA Colorimetric/Fluorometric Assay Kit (Catalog # K739-100) from BioVision, Milpitas, CA, USA. Total antioxidant capacity (TAC) was evaluated using the Rat TAC Elisa kit (Catalog # AMS.E02T0028) from Amsbio, Cambridge, USA. Hepatic triglycerides were quantified using the Rat Triglyceride Elisa Kit (EK720636) from AFG Scientific, Northbrook, USA.

2.6 Western blotting

The expression of Nrf2 protein was assessed using Western blotting. The antibody employed was Nrf2 Antibody (A-10): sc-365949. Hepatic tissue proteins were isolated using the Bio-Rad ReadyPrep™ protein extraction reagent (Catalogue #163-2086). The extracted proteins were then transferred to polyvinylidene difluoride (PVDF) membranes through electrophoresis. After blocking the membranes with 5% non-fat milk in Tris-buffered saline with Tween-20 (TBST), they were incubated overnight at 4°C with the Nrf2 antibodies. Peroxidase-conjugated secondary antibodies were applied, and a chemiluminescent substrate system was used to visualize the protein bands. The signal intensity of the target proteins

was normalized to β-actin, a housekeeping protein, and compared to control samples using image analysis software on the ChemiDoc MP system.

2.7 Histopathological examination and Oil Red O staining

Samples of fresh liver tissue were preserved by fixation in 10% neutral buffered formalin for a 24-hour period. Following this fixation process, tissues were subjected to xylol clearing, paraffin embedding, and a stepwise dehydration procedure using graded concentrations of ethyl alcohol. Specimens were then blocked and sectioned into 5µm-thick slices. These sections underwent hematoxylin and eosin staining, preparing them for detailed microscopic examination **[27].**

Utilizing Oil Red O stain, lipid composition of liver tissue was ascertained. After washing 8–10 µm frozen liver slices with 60% isopropanol, they were stained for 10 minutes with Oil Red solution and then counterstained with hematoxylin. An Olympus microscope was used to take pictures.

3. Statistical analysis

Statistical analysis was performed using SPSS for Windows (Version 20.0; SPSS Inc., Chicago, Illinois). One-way analysis of variance (ANOVA) was used to assess the significance of differences across several groups. In the event of a significant difference, the LDS post hoc test was used to analyze the differences within each group. Results are shown as the mean ± standard deviation (SD) of the mean. A *p*-value of 0.05 or less was deemed significant. The 2-tailed Pearson's correlation coefficient (r) was employed to examine the correlations among asprosin, serum TG, hepatic TG, HDL, HOMA-IR, QUICKI, MDA, TAC, and Nrf2 levels. A *p*-value of 0.05 or less was considered statistically significant.

4. Results:

4.1 CoQ10, exercise, or their combinations attenuate HFD-induced serum dyslipidemia, hepatic steatosis and associated liver dysfunction

According to our findings, feeding on a high-fat diet (HFD) led to dyslipidemia, as seen by a substantial increase in serum TG, TC, and LDL. In contrast, HFD group's HDL levels were considerably lower than those of controls (*P*-value < 0.05). In contrast to HFD group, CoQ10 supplementation and/or exercise substantially reduced levels of blood TG, TC, and LDL and increased levels of HDL in HFD + CoQ10 group, HFD + EX group, and HFD + EX + CoQ10 group (*P*-value < 0.05). Furthermore, when comparing HFD + EX + CoQ10 group to HFD + CoQ10 group and HFD + EX group, there was a substantial rise in HDL levels and a remarkable significant drop in serum TG, TC, and LDL (*P*-value < 0.05) **(Table 1)**.

Moreover, Hepatic TG levels in HFD rats were considerably greater ($P < 0.05$) than in controls, in accordance with changes in serum lipids. In contrast, there was a noteworthy drop in hepatic TG (*P*-value < 0.05) in HFD + CoQ10, HFD + EX, and HFD + EX + CoQ10 groups as compared to HFD group. Furthermore, hepatic TG significantly decreased in HFD + EX + CoQ10 group (*P*-value < 0.05) compared to HFD + CoQ10 group and HFD + EX group **(Table 1).**

Additionally, histopathological evaluation of hepatic tissue showed normal hepatocytes arranged

in single-cell cords with average central vein and average intervening blood sinusoids in controls **(Fig 1a).** On other hand, HFD group revealed congested portal veins and marked macro- and micro-vesicular steatosis of hepatocytes **(Fig 1b)**. In addition, lipid accumulation was assessed using oil red O staining. HFD-fed group showed marked staining for oil red in hepatocytes **(Fig. 2b)** in relation to controls which showed negative staining of hepatocytes for oil red **(Fig 2a)**. All of these findings both at biochemical and histopathological levels indicated development of hepatic steatosis. In addition, treatment with CoQ10, exercise or both improved histopathological changes **(Fig 1c, 1d and 1e)** respectively, and lipid droplets accumulation induced by HFD was ameliorated **(Fig 2c, 2d and 2e)** respectively.

Moreover, HFD group demonstrated a significant elevation in serum ALT and AST levels compared to balanced diet controls ($P < 0.05$), indicating impaired hepatic function in HFD-fed subjects. However, these biomarkers (AST and ALT) were significantly decreased in HFD + CoQ10 group, HFD + EX group, and HFD + EX + CoQ10 group when compared to HFD group ($P < 0.05$). Also, there was a significant decrease in AST and ALT levels in HFD + EX + CoQ10 group when compared with HFD + CoQ10 group and HFD + EX group ($P < 0.05$) **(Table 1)**. Collectively, our results indicated a rat model of HFD-induced NAFLD and that CoQ10 and / or exercise could attenuate its progression and improve liver function.

	Control group	**HFD group**	**HFD + Exercise group**	**HFD + CoQ10 group**	**HFD + Exercise + CoQ10 group**
AST (IU/L)	46.18 ± 2.61	76.53 ± 3.88*	55.48 ± 3.86$^{*, **}$	55.55 ± 3.80$^{*, **}$	48.70 ± 1.95$^{**, \#, \#\#}$
ALT (IU/L)	41.38 ± 2.73	71.01 ± 3.26*	53.21 ± 3.52$^{*, **}$	54.20 ± 3.28$^{*, **}$	48.80 ± 0.93$^{*, **, \#, \#\#}$
TG (mg/dl)	102.33 ± 4.18	243.83 ± 12.89*	178.50 ± 8.34$^{*, **}$	165.17 ± 4.83$^{*, **, \#}$	139.50 ± 6.47$^{*, **, \#, \#\#}$
LDL (mg/dl)	38.17 ± 1.47	74.00 ± 3.35*	63.33 ± 2.58$^{*, **}$	59.67 ± 3.33$^{*, **, \#}$	50.50 ± 2.43$^{*, **, \#, \#\#}$
HDL (mg/dl)	49.83 ± 3.43	20.67 ± 3.50*	37.67 ± 1.97$^{*, **}$	39.50 ± 1.87$^{*, **}$	46.33 ± 3.14$^{*, **, \#, \#\#}$
TC (mg/dl)	116.33 ± 5.47	244.17 ± 9.70*	180.83 ± 11.58$^{*, **}$	176.17 ± 4.96$^{*, **}$	155.00 ± 4.60$^{*, **, \#, \#\#}$
Hepatic TG	0.53 ± 0.26	2.20 ± .68*	1.47 ± 0.40$^{*, **}$	1.55 ± 0.29$^{*, **}$	0.89± 0.10$^{**, \#, \#\#}$

Data are represented as Mean ± SD., n=6. $P < 0.05$ is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.*: $P< 0.05$ vs. Control group; **: $P< 0.05$vs. HFD group, # $p< 0.05$ vs. HFD + Exercise group, ## $p< 0.05$ vs. HFD + Exercise + Co-Q10 group. HFD, high fat diet.CoQ10, coenzyme Q 10.AST, aspartate aminotransferase.ALT, alanine aminotransferase.TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

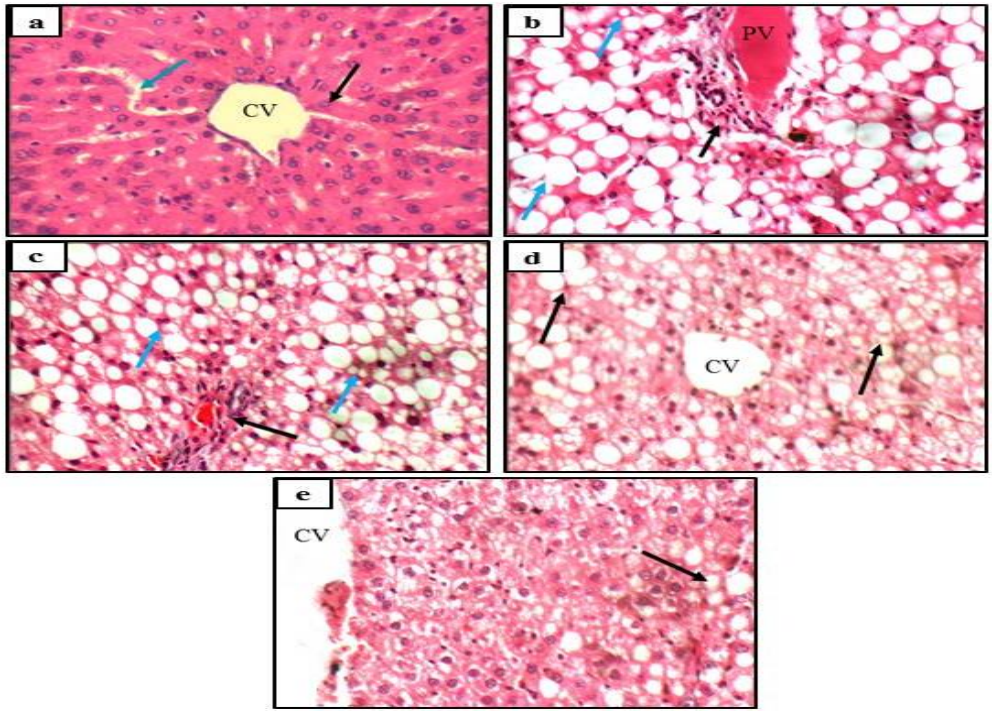

Fig.1: Photomicrograph from liver of experimental groups. (1a) Control group shows average central vein (CV) and average hepatocytes arranged in single-cell cords (black arrow) with average intervening blood sinusoids (blue arrow). (1b) HFD group shows portal tracts (black arrow) with mildly congested portal veins (PV), and marked macro- and micro-vesicular steatosis of hepatocytes in peri-portal area (blue arrow). (1c) HFD + Exercise group shows portal tracts with average portal veins (black arrow), and moderate macro- and micro-vesicular steatosis of hepatocytes in peri-portal area (blue arrow). (1d) HFD + Co-Q10 group showing average central veins (CV) with moderate macro- and micro-vesicular steatosis of hepatocytes in peri-venular area (black arrow). (1e) HFD + Exercise + Co-Q10 group reveals mildly dilated central veins (CV) with mild macro- and micro-vesicular steatosis of hepatocytes in peri-venular area (black arrow) (H&E X 400).

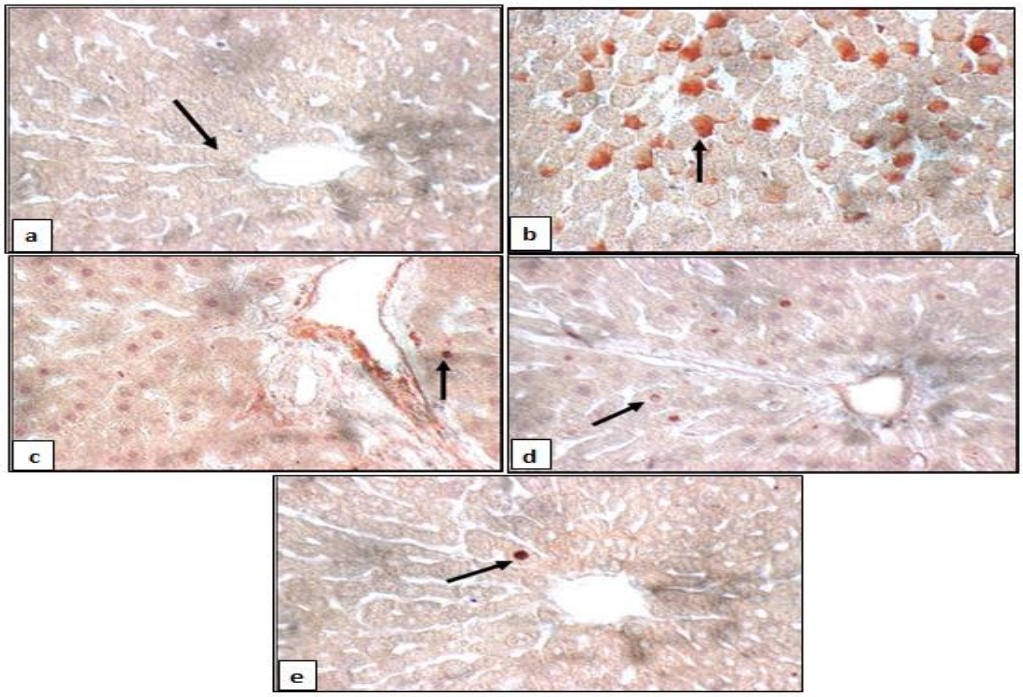

Fig. 2: Oil red O staining of liver tissue in different experimental groups. (2a): Control group showing negative staining for oil red in hepatocytes (black arrow). **(2b):** HFD group showing marked staining for oil red in hepatocytes (black arrow). **(2c):** HFD + Exercise group showing moderate staining for oil red in hepatocytes (black arrow**). (2d):** HFD + Co-Q10 group showing mild staining for oil red in hepatocytes (black arrow). **(2e):** HFD + Exercise + Co-Q10 group showing mild staining for oil red in hepatocytes (black arrow) (Oil red x 400).

4.2 CoQ10, exercise or their combination ameliorate insulin resistance and improved its sensitivity

Notably, QUICKI (insulin sensitivity marker) significantly decreased while insulin, blood glucose, and HOMA-IR significantly increased in HFD fed group in comparison to balanced diet fed group (P < 0.05), ensuring HFD-induced IR. Conversely, in comparison to HFD group, these changes in insulin, blood glucose, HOMA-IR, and QUICKI levels were dramatically reversed by CoQ10 supplementation or exercise. Additionally, HFD + EX + CoQ10 group showed a notable increase in QUICKI and a notable decrease in insulin and serum glucose as well as HOMA-IR (P-value < 0.05) when compared to HFD + CoQ10 group and HFD + EX group. These results suggest that combination therapy involving CoQ10 and exercise has a greater protective effect **(Table 2).**

Table (2): Fasting blood glucose level, insulin, HOMA-IR, and QUICKI levels in the experimental groups

	Control group	HFD group	HFD + Exercise group	HFD + Co-Q10 group	HFD + Exercise + Co-Q10 group
Glucose (mg/dl)	83.83 ± 8.28	176.50 ± 6.72*	142.33 ± 6.28$^{*, **}$	141.00 ± 3.41$^{*, **}$	132.67± 5.24$^{*, **, \#, \#\#}$
Insulin (µu/ml)	1.37 ± 0.43	20.17 ± 3.19*	10.17 ± 1.60$^{*, **}$	8.47 ± 1.12 $^{*, **}$	6.03 ±0 .89$^{*, **, \#, \#\#}$
HOMA-IR	0.29 ± 0 .10	8.77 ± 1.32*	3.57 ± 0.54$^{*, **}$	2.95 ±0 .38 $^{*, **}$	1.97 ± 0.25$^{*, **, \#, \#\#}$
QUICKI	0.49 ± .04	0.28 ± 0.01*	0.32 ± 0.01*, **	0.33 ± 0.01*, **	0.35 ± 0.01*, **, #

Data are represented as Mean ± SD., n=6. P< 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.*: P< 0.05 *vs.* Control group; **: P< 0.05*vs.* HFD group, #p< 0.05 *vs.* HFD + Exercise group, ## p< 0.05 *vs.* HFD + Exercise + CoQ10 group. HFD, high-fat diet; CoQ10, coenzyme Q 10; HOMA-IR: Homeostasis model assessment insulin resistance; QUICKI, quantitative insulin-sensitivity check index.

4.3 CoQ10, exercise or their combination alleviate HFD-induced oxidative stress:

Rats on a HFD had considerably higher levels of lipid peroxidation marker (MDA) in their hepatic tissue; whereas their hepatic TAC levels were considerably lower in comparison to controls (P-value < 0.05). In comparison to HFD group, HFD + CoQ10, HFD + EX and HFD + EX + CoQ10 groups all dramatically reduced lipid peroxidation as they significantly decreased hepatic MDA and significantly raised hepatic TAC, indicating that exercise, CoQ10, both boosted antioxidants. Additionally, when comparing HFD + EX + CoQ10 group to HFD + CoQ10 group and HFD + EX group, combination of CoQ10 plus exercise resulted in a substantial drop in MDA and a significant rise in TAC (P-value < 0.05) **(Table 3).**

4.4 CoQ10, exercise or combination of both mitigate the HFD-induced liver injuries by regulating the asprosin/Nrf2 Pathway:

As compared to controls, our results showed a substantial rise in asprosin levels in HFD fed group (P < 0.05). Interestingly there was a significant decrease in asprosin level in HFD + CoQ10 group, HFD + EX group and HFD + EX + CoQ10 group (P-value < 0.05) when compared to HFD group. In addition, combination of CoQ10 and exercise in HFD + EX + CoQ10 significantly decreased asprosin level when compared with HFD + CoQ10 group and HFD + EX group *(*P-value < 0.05) **(Table 3)**. On other hand, HFD resulted in a significant decrease in hepatic Nrf2 protein expression in HFD group (P < 0.05) when compared by controls. While there was a

significant increase in Nrf2 protein expression in hepatic tissues of HFD + CoQ10 group, HFD + EX group and HFD + EX + CoQ10 group (*P*-value < 0.05) when compared with HFD group. Additionally, when comparing HFD + EX + CoQ10 to HFD + EX group and HFD + CoQ10 group, there was a substantial increase in Nrf2 protein expression (*P*-value < 0.05) **(Fig. 3).**

4.5 Correlation between serum asprosin level and serum TG, hepatic TGs, HDL, HOMA-IR, QUICKI, MDA, TAC, and Nrf2 levels:

Serum asprosin and other indicators showed a significant correlation (*P*-value < 0.05). It had a positive correlation with serum TG (r = 0.974), hepatic TG content (r = 0.801), HOMA-IR (r = 0.954), and MDA (r = 0.903) levels. Conversely, a negative correlation with HDL (r = -0.928), TAC (r = - 0.864), QUICKI (r= -0.818) and Nrf2 (r = - 0.827) **(Fig. 4).**

Table (3): Serum asprosin and hepatic TAC andMDA levels in the experimental groups

	Control group	HFD group	HFD + Exercise group	HFD + Co-Q10 group	HFD + Exercise + Co-Q10 group
Asprosin (pg/ml)	55.83 ± 3.87	146.17± 5.42*	105.33 ± 6.15$^{*, **}$	94.67 ± 2.42$^{*, **,\#}$	75.50 ± 5.24$^{*, **, \#, \#\#}$
TAC (ng/mg protein)	3.90 ± 0.48	1.56 ± 0.52*	2.33 ± 0.36$^{*, **}$	2.35 ± 0.40 $^{*, **}$	2.88± 0.26$^{*, **, \#, \#\#}$
MDA (nmol/mg protein)	17.95 ± 1.10	36.65 ± 5.84*	26.63 ± 4.18$^{*, **}$	26.08 ± 3.87$^{*, **}$	19.76 ± 1.55$^{**, \#, \#\#}$

Data are represented as Mean ± SD., n=6. *P* < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. *: *P*< 0.05 vs. Control group; **: *P*< 0.05vs. HFD group, # *p*< 0.05 vs. HFD + Exercise group, ## *p*< 0.05 vs. HFD + Exercise + Co-Q10 group.HFD, high fat diet.CoQ10, coenzyme Q 10.TAC, total antioxidant capacity. MDA, malondialdehyde.

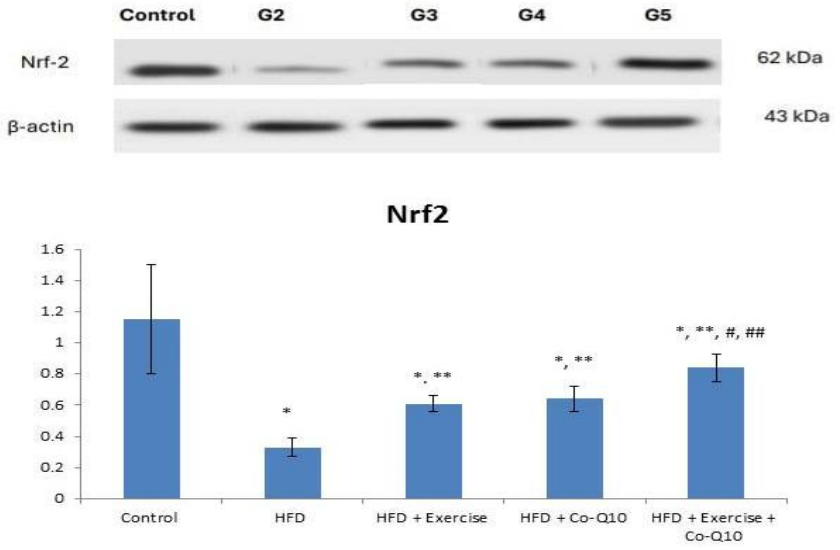

Fig. 3: Western blotting with densitometricanalysis of Nrf2 protein expression in the experimental groups

Data are represented as Mean ± SD., n=6. *P*< 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.*: *P*< 0.05 *vs*. Control group; **: *P*< 0.05*vs*. HFD group, #*p*< 0.05 *vs*. HFD + Exercise group, ## *p*< 0.05 *vs*. HFD + Exercise + CoQ10 group.HFD; high-fat diet, CoQ10, coenzyme Q 10, Nrf2; nuclear factor erythroid 2-related factor 2.

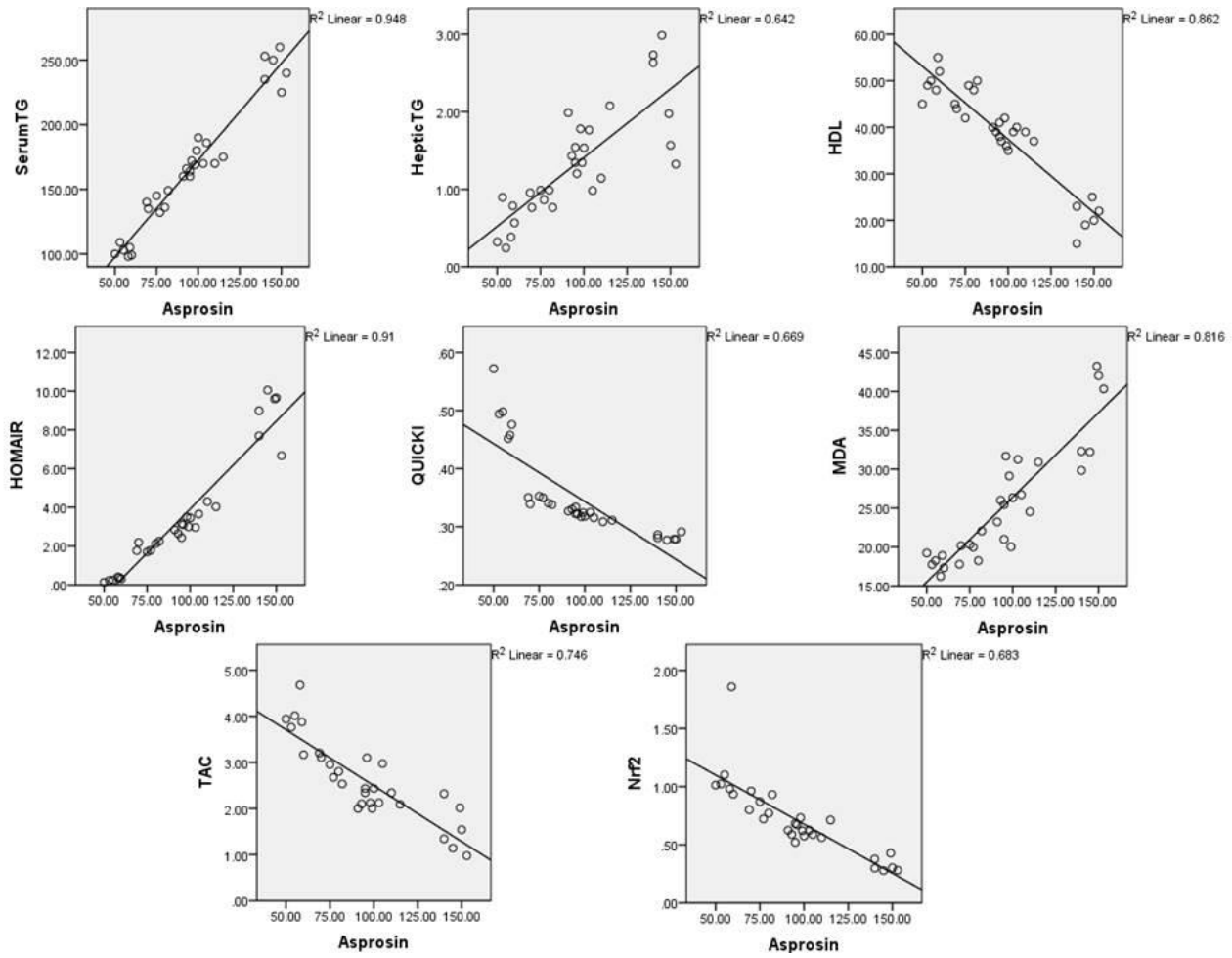

Fig. 4: Correlation between serum asprosin level and serum TG, hepatic TG, serum HDL, HOMA-IR, QUICKI, and hepatic MDA, TAC, and Nrf2 protein levels

It was analyzed using Pearson's correlation coefficient (r) 2-tailed test. A p-value of less than 0.05 was considered statistically significant. HDL; high-density lipoprotein, HOMA-IR; homeostasis model assessment- insulin resistance, MDA; malondialdehyde, Nrf2; nuclear factor erythroid 2-related factor 2, QUICKI, quantitative insulin-sensitivity check index. TAC, total antioxidant capacity; TG; triglyceride.

5. Discussion

NAFLD is presently identified as a liver injury caused by metabolic stress, with hepatocyte steatosis serving as defining diagnostic feature. A considerable amount of research has established that excessive caloric intake is a major risk factor for onset of NAFLD **[28]**. Hence, in this study, HFD was given to construct an in vivo model of NAFLD. The primary findings of present study include that HFD-fed group compared to control exhibited significant dyslipidemia presented by marked ascending in serum TG, LDL and TC with notable decrease in HDL. This was accompanied by an elevation in hepatic TGs content at biochemical level with macro- and micro-vesicular hepatocytic steatosis and portal congestion as evidenced by histopathological examination. Oil red O staining, also demonstrated marked lipid buildup within hepatocytes. All of this eventually caused damage and impaired liver function. Serum AST and ALT levels that are vital benchmarks for evaluation of liver injury were significantly augmented. According to earlier reports by **Wang et al. [29] and Farage et al. [30],** these findings confirm emergence of hallmark features of NAFLD following administration of HFD.

Under HFD conditions, absorbed lipid was circulating as free fatty acids (FFA) and TG-rich chylomicrons. Approximately 20% are taken by liver, where they may undergo β-oxidation-mediated metabolism, be combined with apolipoprotein B and released into bloodstream as LDL, or be retained in lipid droplets after being esterified to TG **[31]**.

The present study showed an observed higher expression of asprosin in rats with induced NAFLD *vs.* control. Additionally, remarkable significant positive correlations were found between serum asprosin and TGs both serum and hepatic levels in addition to LDL, while a significant negative correlation with HDL levels. These findings denote hepato-adipogenic effect of augmented asprosin. In line with this, plenty of animal and clinical studies demonstrated that serum asprosin expression is amplified in NAFLD **[32, 33]**.

Additionally, in comparison to controls, HFD-induced NAFLD was also associated with higher insulin, blood glucose, and HOMA-IR and lower QUICKI (an insulin sensitivity marker). Furthermore, asprosin was shown to have an impressive positive correlation with HOMA-IR and a significant negative correlation with QUICKI index. Asprosin, a recently identified glucogenic adipokine, has been implicated as a crucial contributor to insulin resistance development and modulation of glucose metabolism within liver. At liver, asprosin causes a glucogenic effect through a cyclic AMP-dependent protein kinase regulation of glycogen and lipid metabolism **[6].** These results pinpoint to close relation between asprosin, NAFLD, and IR in a positive feedback manner. In line with this, a recent published study reported that asprosin was overexpressed in NAFLD-induced animals fed a HFD and when asprosin was neutralized, a drop of blood glucose level was observed **[34].**

Oxidative stress as well, has been demonstrated to play an integral role in NAFLD pathogenesis and progression **[35]**. Our data revealed lipid peroxidation indicator (MDA) was significantly increased in HFD-fed rats, whereas TAC was decreased. Also, serum asprosin level was significantly positively correlated with hepatic MDA while negatively correlated with hepatic TAC levels. These findings matched with those of **Masarone et al. [36], Li et al. [37]**, who attributed this to an excessive buildup of TGs that exacerbate production of ROS that disrupting redox homeostasis. An initiating factor for NAFLD is aberrant hepatocyte lipid accumulation that contributes to release of ROS causing lipid peroxidation and diminishing of antioxidants that further provoking liver tissue injury **[38].** In addition, IR can also trigger release of ROS by up-regulating microsomal lipid peroxidation and down-regulating mitochondrial β oxidation.

More intriguingly, Nrf2 protein expression was observed to be reduced in HFD group compared to control that further confirmed the decline in TAC. This observation is consistent with findings of **Vomhof-DeKrey et al. [39],** who reported a reduction in hepatic mRNA expression of Nrf2 and its downstream targets in HFD group. Additionally, studies have shown decreased Nrf2 levels in human liver tissues and diabetic animals given a high-fat diet **[40, 41].** Also, in current work, a significant negative correlation between

serum asprosin level and hepatic Nrf2 was documented. This predisposes for lipogenesis secondary to inhibition of browning of adipose tissue and reduced energy expenditure. **Miao et al**. **[42]** has concluded that asprosin influences Nrf2-supressing mechanism to adversely regulate browning and increased lipid accumulation in adipose tissue.

Up till now, no pharmacological treatment has been approved by United States Food and Drug Administration for NAFLD, and aside from oxidative stress, underlying mechanisms contributing to pathogenesis of this condition remain incompletely understood **[34].** At this time, targeting restoration of redox homeostasis and lipid metabolism in addition to asprosin decline may be considered as an effective strategy.

In current investigation, coenzyme Q10 supplementation and/or exercise training exerted a hepato-protective impact against HFD-induced NAFLD changes with better effect of their combination suggesting a synergistic effect. Our data revealed that although of HFD feeding to rats, CoQ10 and /or exercise protecting against serum dyslipidemia, hepatic steatosis both at biochemical and pathological levels with reduction of hepatic injury markers, serum AST and ALT when compared to HFD only fed rats. In addition, there was an improvement of associated IR state as evidenced by decline in HOMA-IR and improved insulin sensitivity indicated by an increase in QUICKI.

These findings were in agreement with earlier observations **[18, 43]** and confirmed hepato-protective glucose homeostatic effect of CoQ10 and exercise.

Regarding conserving lipid metabolism in HFD groups managed by CoQ10 and/or exercise, could be explained by CoQ10's ability to inhibit expression of lipogenesis-linked genes as well as hepatic de novo lipogenesis, which lowers hepatic lipid buildup **[18].** Furthermore, CoQ10 increases fat oxidation and energy expenditure in inguinal white adipose tissue **[44]**. CoQ10 also, has ability to directly scavenging ROS particularly peroxide radicals **[45]**. Concerning underlying exercise-mitigating effect was that exercise restore hepatic lipid metabolism by decreasing acetyl-CoA carboxylase and fatty acid synthesis **[43, 46].**

Nrf2 augmentation in our study was also among underlying ameliorative effect of CoQ10 and/or exercise on NAFLD. In consistent with this concept, our results matched with previous studies which reported that antioxidant effect of Co Q10 *via* Nrf2 up regulation **[47]**. Moreover, exercise prevented HFD-induced reduction in expression of Nrf2 protein in HFD group as previously reported **[48].** In same vein, an additional investigation demonstrated that physical exercise can significantly enhance expression of Nrf2 protein in heart of both young and elderly rats **[49].** In addition to maintenance of redox homeostasis by increasing antioxidants levels and TAC, Nrf2 negatively regulates fatty acid binding protein which results in suppression of hepatocytes' FFAs uptake **[50]**. Also, Nrf2 can suppress lipogenesis and improve mitochondrial function **[51]**.

Asprosin suppressing effect in CoQ10 and/or exercise managed groups was observed in comparison with HFD-induced NAFLD. Researches concerning impact of CoQ10 and exercise on asprosin level remain relatively scarce.

To extent of our current understanding, asprosin-lowering effect of CoQ10 was not explored before. In addition, a recently published work has evidenced that exercise decreased asprosin **[52].** Also, in current work presented, a significant negative correlation between serum asprosin level and Nrf2 was documented. This could be explained by that improvement in oxidative stress status minimizing secretion of asprosin and its bad consequences.

Conclusion

This study shows that CoQ10 or exercise has hepato-protective benefits in a rat model of HFD-induced NAFLD. Rise in serum asprosin levels contributed to development and progression of NAFLD. Mechanisms underlying this protective effect might be linked to reducing dyslipidemia and insulin resistance in addition to anti-oxidative effects, which might potentially be explained by increases in Nrf2 proteins expression in hepatic tissue. Furthermore, there is a greater protective impact when CoQ10 and exercise are combined. Thus, to prevent NAFLD or protect against its progression, CoQ10 supplementation or ingestion of diet-rich sources alone or in combination with exercise would be helpful.

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All authors contribute equally in the study design, collection of samples, data analysis, and manuscript writing.

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