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N-acetyl Cysteine Can Blunt Metabolic and Cardiovascular Effects via Down-regulation of Cardiotrophin-1 in Rat Model of Fructose-induced Metabolic Syndrome

Azza salaheldien Abdelhaffez (azzasalah10@hotmail.com)
Assiut University Faculty of Medicine https://orcid.org/0000-0003-1567-7041
Ebtihal Anwar Abd El-Aziz
Assiut University Faculty of Medicine
Maha Baghdady Tohamy
Assiut University Faculty of Medicine
Asmaa Mahmoud Ahmed
Assiut University Faculty of Medicine

Research

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Abstract

Background

Chronic fructose consumption is associated with development of obesity, insulin resistance (IR) and metabolic syndrome (MS). Cardiovascular diseases are linked to metabolic deregulation observed in MS. N-acetylcysteine (NAC) is a sulfur containing compound of *Allium* plants (such as garlic and onion) that increases intracellular reduced glutathione concentrations, which is an endogenous antioxidant.

Methods

This study investigated the ability of N-acetyl cysteine (NAC) to alleviate the metabolic disorders in fructose-induced MS in male Wistar rats and to examine its protective effect on aortic and cardiac tissues via its influence on cardiotrophin-1 (CT-1) expression. NAC (20 mg/kg b.w./day) was administered to fructose (20% w/v) induced MS animals for twelve weeks.

Results

Chronic fructose consumption increased body weight gain, relative heart weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), IR and associated with metabolic alterations. Histological and immunohistochemical examination revealed aortic stiffness and myocardial degeneration and fibrosis together with increased CT-1 expression. Treatment with NAC improved IR, SBP, DBP and mitigated atherogenic dyslipidaemia and oxidative stress (OS). Additionally, NAC down-regulated CT-1 expression in the heart and aorta. Furthermore, CT-1 expression in the heart and aorta was positively correlated with basal glycemia, final SBP and DBP, total cholesterol, triglycerides, low density lipoproteins and negatively correlated with high density lipoproteins levels.

Conclusion

The findings reported here demonstrated, for the first time, the protective effect of NAC against aortic and myocardial degeneration and fibrosis through down-regulation of CT-1 in fructose induced MS animal model. Also, our results revealed the infallible role of NAC to blunt the cardiometabolic deregulation observed in MS.

Introduction

Metabolic syndrome (MS) is a promptly growing global pandemic, which is associated with a greater threat of numerous chronic pathologies comprising cardiovascular diseases and Type 2 diabetes. It is characterized by a cluster of metabolic abnormalities including elevated fasting glucose, insulin resistance (IR), obesity, atherogenic dyslipidemia, hypertension and low-grade inflammation [1]. One of

the main contributing operator for the development of MS is the diabetogenic food with high fructose content. It is also involved in the development of Type 2 diabetes and cardiovascular diseases [2]. Animals nourished a high-fructose diet develop clinical features of MS such as IR, dyslipidemia and adiposity which may be helpful for evaluating possible curative interventions against MS [3].

N-acetyl cysteine (NAC) is a pleiotropic molecule found in plants of the *Allium* species, especially in the onion [4]. It is the N-acetylated byproduct of the natural amino acid L-cysteine. Due to its molecular structure, it easily gets into the cell where it is acetylated there and converts into L-cysteine. L-cysteine is a glutathione precursor and encourages glutathione formation. Glutathione is an extremely reactive tripeptide, which defends cells against detrimental influences of endogenic or exogenic cytotoxic substances and oxidative radicals, and participates in an endocellular mechanism for the keeping of cellular integrity and functions. Also, the sulfhydryl group (–SH) within the NAC molecule directly scavenges reactive oxygen species (ROS) [5]. NAC is presently used as a mucolytic and in HIV treatment, and it has demonstrated efficacy in chronic obstructive pulmonary disease and in certain conditions of nephropathy [6].

Cardiotrophin-1 (CT-1) is a protein that firstly identified in the supernatant of mouse embryonic corpuscles in 1995. It acquired its name from the capability to induce a hypertrophic response in neonatal cardiac myocytes [7]. It is one of the pro-inflammatory cytokines belonging to the IL-6 cytokine superfamily that has been showed to do a diversity of actions in many organs such as the heart, liver, adipose tissue, and atherosclerotic arteries [8]. Growing evidence showed increased circulating CT-1 levels in humans with obesity [9] and type 2 diabetes [10], advocating that CT-1 may play a pathophysiological role in obesity-related complications.

The aim of this study was to assess the effect of NAC on CT-1 expression in both the heart and aorta in high-fructose fed rats with MS. Also, to elucidate the potential alleviating effect of NAC on hyperlipidemia, hyperglycemia, IR and OS in those rats.

Material And Methods

Animals and diets

Thirty two healthy adult male Wistar rats with a body weight of 160–190 gm were supplied by the animal's house facility, Faculty of Medicine, Assuit University. The rats were housed in stainless steel wire-bottomed cages in controlled temperature (20 ± 5 °C), 12:12 h dark-light cycle. The rats were acclimatized to the housing facility for 7 days with free access to standard laboratory pellet food and water prior to the experiment. The experimental protocols were approved by Animal Ethics Committee of Assiut University (Institutional review board (IRB) approval NO: 17100802) and in accordance with the internationally accepted principles for Guide for the Care and Use of Laboratory Animals.

Experimental Design

Rats were randomly divided into four groups of eight animals each including:

1. Control: rats in this group received standard diet and tap water.
2. NAC: rats in this group received standard diet, tap water and a daily dose of NAC (20 mg/kg/day dissolved in distilled water and given by oral gavage for 12 weeks [11].
3. MS: rats in this group received standard diet and tap water supplemented with 20% (w/v) fructose for 12 weeks.
4. MS + NAC: rats in this group received standard diet, tap water supplemented with 20% (w/v) fructose and a daily dose of NAC as described above for 12 weeks.

All experimental groups were fed ad libitum with the standard laboratory chow diet.

Fructose-induced Metabolic Syndrome

Fructose was used in this study to develop a rat model of MS. Fructose drinking water (FDW) was freshly prepared every day. To prepare 20% of FDW, 20 g of fructose was diluted in 100 ml of tap water [12]. The water bottles were covered with aluminium foil to prevent fermentation. The FDW was given every day for 12 weeks as ad libitum to the rats to induce MS.

Reagents

All chemicals used in the experiments were of analytical grade. N-acetylcysteine (NAC, C5H9NO3S), D-fructose and D-glucose were purchased from Sigma (St. Louis, MO, USA).

Body Weight And Body Weight Gain

Body weight was measured weekly throughout the whole period of the study using electronic weighing scale. Then, Percentage body weight gain was calculated as: (Body weight on sacrifice day (g) – initial body weight)/ initial body weight × 100 [13].

Fasting Blood Glucose Measurement

It was done weekly using blood samples obtained from the rats' tail using a glucometer after incision of the distal part of the tail using Salut blood glucose meter (FIA Biomed glucometer, Germany).

Blood Pressure Measurement

Blood pressure was measured by the tail-cuff method in all groups of rats using LE5001 Non Invasive Blood Pressure Meter with sphygmomanometer technique (Panlab Harvard Apparatus, Barcelona, Spain)

at 1st, 3rd, 6th, 9th, 12th week. Three readings were taken, then, the average reading was calculated and taken as the final reading [12]

Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT)

Oral glucose and insulin tolerance tests were performed during weeks 11–12. To perform OGTT, animals were fasted overnight and then received a glucose solution (1 g/ kg body weight) by oral gavage. Blood glucose levels were measured before (baseline) and at 15, 30, 60, 90 and 120 min after glucose administration. For ITT, regular insulin (Humulin U-100; Lilly, Indianapolis, IN, USA) in a saline solution (0.5U/kg) was intraperitoneal injected following a 12 h fast. Blood glucose levels were measured immediately before the insulin injection (time 0) and at 15, 30, 60, 90 and 120 min after insulin injection. Same groups of animals were used for OGTT and ITT with a time interval of one week. The trapezoidal integration was used to calculate areas under curve (AUC) [14].

Estimation of serum insulin levels, insulin resistance and sensitivity indices

Serum insulin levels were measured using Ray Bio Rat insulin ELISA kit (Ray Biotech, Norcross, GA, USA). The IR was estimated by the homeostasis model assessment of IR (HOMA-IR) [15], and insulin sensitivity was estimated by quantitative insulin-sensitivity check index (QUICKI) [16], according to the following formulae:

HOMA-IR = insulin (μIU/mL) × glucose (mmol/L)/22.5

QUICKI = 1/log fasting insulin level (μU/ml) + log fasting blood glucose (mg/dl).

Collection Of Blood And Tissue Samples

At the end of the treatment period and after an overnight fast period, blood samples were taken from the tail vein then the animals were weighed and euthanized. Blood samples rested for a short period of time, centrifuged (1000 g, 10 min) and stored at − 20 °C for further biochemical analyses. The heart and aorta was rapidly removed, washed in cold saline solution, placed in qualitative filter paper for excess liquid removal, and the heart was weighed. The heart and aorta then fixed in 10% formalin for histopathological and immunohistochemical examination. Relative heart weight was calculated according to the following equation:

Relative heart weight = absolute heart weight (g) × 100/ body weight of rat on sacrifice day (g) [13].

Biochemical Analysis

Assessment of tissue levels of Oxidative stress markers

Serum malondialdehyde (MDA) level (mmol/L), marker of lipid peroxidation production and the total antioxidant status were assayed in the serum by spectrophotometric measurement using a commercial kit (Biodiagnostic, Egypt) according to the manufacture instructions.

Estimation of lipid profile and athrogenic index

Serum lipid profile, including total cholesterol, triglycerides, low-density lipoprotein- cholesterol (LDL-C) and high-density lipoprotein-cholesterol(HDL-C) were estimated using a spectrophotometer analysis. Kits were purchased from the Egyptian Company for Biotechnology.The atherogenic index was calculated as demonstrated by Takasaki [17], atherogenic index = (total cholesterol – HDL cholesterol)/ HDL-cholesterol.

Histopathological examination:

Left ventricular and thoracic aortic tissues were fixed in 10% formaldehyde, then dehydrated with alcohol series and embedded in paraffin wax by routine protocols. 5μm thin sections were prepared and stained with Haematoxylin and Eosin (H&E) stain, and Masson's Trichome (MT) stain (to assess connective tissue deposition). For the aortic tissues, the thickness of the tunica media (TM) was evaluated at a magnification of x400 using a digital image analysis system (Leica Qwin 500; Leica, Cambridge, UK).

Immunohistochemistry

For immunohistochemical staining, sections were deparaffinized and rehydrated. Blocking of the endogenous peroxidase activity was done by 3% hydrogen peroxide. Then, sections were heated in 10 mM citrate buffer (pH 6.0) with microwave at 80 °C for 15 min for antigen retrieval. Primary antibody (CT-1, Catalog # PA5-71926, dilution 1:100, Thermo-scientific corporation, Fremont, CA, USA) was used. The slides were incubated overnight at room temperature. Secondary staining kits were used according to the manufacturer's instructions (Thermo scientific corporation Fremont, CA, USA). Cytoplasmic staining was considered positive. The staining intensity of CT-1 was scored as the following (0, no staining; 1, mild intensity; 2, moderate intensity; and 3, maximum intensity).

Statistical analysis

The results were expressed as the mean ± standard error of the mean. Statistical analysis of the difference between groups was done using the one-way analysis of variance (ANOVA) and two-way ANOVA followed by Tukey's test as a post hoc analysis for the one-way method and Bonferroni's test as a post hoc analysis for the two-way method. Pearson's correlation coefficient was used to analyze associations between quantitative variables. All statistics were carried out using Graph Pad Prism software version 7 (Graph Pad; San Diego CA, USA).

Results

Effect of N-acetyl cysteine (NAC) administration on physiological variables.

The percentage of body weight gain and relative heart weight were significantly higher in fructose fed group when compared to the control group. In comparison with the MS group, the percentage of body weight gain and relative heart weight of MS + NAC group showed significant decrement (Table 1).

Table 1
Physiological variables of the studied groups.

	Control	NAC	MS	MS + NAC
Body weight gain (%)	27.56 ± 0.52	30.11 ± 1.11	79.48 ± 3.2***	56.73 ± 4.37***,###
Relative heart weight (%)	0.05 ± 0.01	0.07 ± 0.02	0.22 ± 0.02***	0.03 ± 0.01###
Final SBP (mmHg)	102.65± 7.37	105.3 ± 8.85	220 ± 12.48***	142 ± 10.78***,###
Final DBP (mmHg)	74.45± 5.98	70.57 ± 4.87	155.67 ± 6.56***	128.68 ± 4.2***,##
Data are presented as mean ± SE (n = 8 rats in each group), SBP; Systolic blood pressure, DBP; Diastolic blood pressure. Data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests.				
*** Significantly different vs. control group (P < 0.001).				
## Significantly different vs. MS group (P < 0.01).				
### Significantly different vs. MS group (P < 0.001).				

Also, MS group showed remarkable increase in final SBP level and final DBP compared to the control group. Co-administration of NAC with fructose to the animals exhibited significant reduction in final SBP and final DBP in comparison with the MS group (Table 1).

The present data showed gradual and significant increase in body weight of MS induced animals compared to the controls starting from the 6th week, 9th week till the 12th week. In comparison with the MS group, the NAC treated MS induced animals showed a significant decline in their body weight in the 12th week (Fig. 1A).

In comparison to the controls, SBP showed a significant increase in MS induced animals began from the 3rd week, the 6th week, the 9th week on ward till the 12th week. NAC treatment to MS induced animals demonstrated a remarkable decrease in SBP level when compared with the MS group starting from the 9th week till the 12th week (Fig. 1B).

DBP showed a remarkable increase in the MS group in comparison with the control group starting from the 3rd week onward, the 6th week, the 9th week, till the 12th week. With NAC treatment to MS induced animals, the DBP was significantly lower than their corresponding in the MS group starting from the 3rd week, the 6th week, the 9th week, till the 12th week (Fig. 1C).

Effect of N-acetyl cysteine (NAC) administration on blood glucose homeostatic variables.

At the end of the experiment, fasting blood glucose and serum insulin levels were significantly elevated in MS group compared to control group. Treatment with NAC to MS induced animals resulted in remarkable reduction of blood glucose and serum insulin levels compared to MS group (Table 2).

Table 2
Blood glucose homeostatic variables of the studied groups.

	Control	NAC	MS	MS + NAC
Glucose(mg/dl)	76.38 ± 2.55	78.58 ± 2.83	189.1 ± 15.71***	134.1 ± 11.03**,##
Insulin(µIU/ml)	6.1 ± 0.35	6.56 ± 0.25	14.44 ± 0.48 ***	9.94 ± 0.55***,###
HOMA-IR	1.15 ± 0.09	1.26 ± 0.04	6.77 ± 0.7***	2.61 ± 0.14*,###
QUICKI	0.38 ± 0.004	0.37 ± 0.002	0.29 ± 0.004***	0.32 ± 0.004***,###
Data are presented as mean ± SE (n = 8 rats in each group) HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin-sensitivity check index. Data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test.				
*Significantly different vs. control group (P < 0.05).				
** Significantly different vs. control group (P < 0.01).				
*** Significantly different vs. control group (P < 0.001).				
##Significantly different vs. MS group (P < 0.01).				
### Significantly different vs. MS group (P < 0.001).				

To assess the effect of NAC on IR and sensitivity, HOMA-IR and QUICKI were determined. MS rats exhibited a significant increase in HOMA-IR and decrease in QUICKI compared to controls. The treatment with NAC produced a significant amelioration in insulin sensitivity as evident by its effect on HOMA- IR and QUICKI (Table 2).

At 11–12 weeks of the experiment, rats were submitted to OGTT and ITT. Figure 2A& B demonstrated the time course of blood glucose after high oral glucose intake during OGTT. By comparing the AUC, there was a significant trend towards a better tolerance to glucose in NAC treated rats who administered fructose compared to rats administered fructose only. For ITT, the insulin sensitivity was significantly higher in MS + NAC animals than in MS rats (Fig. 2C& D).

Effect of N-acetyl cysteine (NAC) administration on oxidative stress variables

Serum level of MDA was remarkably higher in the MS group compared to the control group. Co-administration of NAC and fructose led to a significant reduction in MDA level in comparison with the MS

group. Meanwhile, MS animals showed an obvious decrease in TAC level compared to the control animals. NAC supplementation to fructose induced MS rats resulted in significant rise in TAC level in comparison with MS group (Fig. 3).

Effect of N-acetyl cysteine (NAC) administration on serum Lipid profiles and atherogenic index.

After 12 weeks of high fructose intake, serum levels of total cholesterol, triglycerides and LDL-C were significantly increased compared to control animals. NAC treatment to MS induced animals displayed reduction in total cholesterol, triglycerides and LDL-C levels compared to MS group. Also, chronic fructose consumption led to a significant drop in HDL-C compared to controls. NAC treatment to MS induced animals showed significant increase in serum HDL-C level in comparison with MS group. Furthermore, atherogenic index in MS induced animals exhibited a remarkable increase compared to control animals. Concomitant administration of fructose and NAC led to significant decrement in atherogenic index in comparison with the MS group (Fig. 4).

Histopathologic Examination Of The Aorta

By H&E staining, no pathologic changes were detected in the control group (Fig. 5A) and the NAC group (Fig. 5B). Significant increase in the TM thickness was observed in the MS group (Fig. 5C) as compared to the control group. However, in the MS + NAC group (Fig. 5D), the TM thickness was significantly lower compared to the MS group and significantly higher compared to the control group (Table 3).

Table 3
Tunica media thickness in the aorta and cardiotrophin-1 expression in the aorta and the cardiac tissue of the studied groups.

	Control	NAC	MS	MS + NAC
Tunica media thickness (µm)	77.09 ± 3.24	77.89 ± 3.16	$219.5 \pm 19.27^{***}$	$123 \pm 3.13^{*,\#\#\#}$
Aortic CT-1	1 ± 0	1 ± 0	$2.63 \pm 0.18^{***}$	$1.38 \pm 0.18^{\#\#\#}$
Cardiac CT-1	1 ± 0	1 ± 0	$2.75 \pm 0.16^{***}$	$1.75 \pm 0.25^{**,\#\#\#}$
Data are presented as mean ± SE (n = 8 rats in each group). Data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests.				
* Significantly different vs. control group (P < 0.05).				
** Significantly different vs. control group (P < 0.01).				
*** Significantly different vs. control group (P < 0.001).				
### Significantly different vs. MS group (P < 0.001).				

MT stain revealed no obvious connective tissue deposition in the control group (Fig. 5E) and the NAC group (Fig. 5F). However, increased connective tissue deposition in the TM of the aorta was observed in the MS group (Fig. 5G). The connective tissue deposition in the MS + NAC group was lesser than in the MS group (Fig. 5H).

Histopathologic Examination Of The Cardiac Tissues

By H&E staining, no pathologic changes were detected in the control (Fig. 6A) and the NAC groups (Fig. 6B). However, cardiac tissues of the MS group showed vacuolar degeneration of the cardiomyocytes (Fig. 6C), mononuclear inflammatory cellular infiltrate (Fig. 6D) and areas of interstitial hemorrhage (Fig. 6E). Improvement of all these histopathologic changes was noted in the MS + NAC group (Fig. 6F).

MT stain revealed no interstitial fibrosis in the control group (Fig. 6G) and the NAC group (Fig. 6H). Meanwhile, in the MS group (Fig. 6I) increased interstitial fibrosis (Fig. 6J) and connective tissue deposition in the wall of the blood vessels (Fig. 6K) were observed. No interstitial fibrosis was detected in the MS + NAC group (Fig. 6L).

Immunohistochemical Expression Of Ct-1 In Aorta

CT-1 protein expression was detected in endothelial cells and smooth muscle cells in the intima and media of the aorta. Immunohistochemistry revealed weak cytoplasmic positivity of CT-1 protein in both control (Fig. 7A) and NAC (Fig. 7B) groups. In the MS group (Fig. 7C), significantly higher CT-1 expression was observed compared to the control group. Decreased CT-1 expression was observed in the MS + NAC (Fig. 7D) compared to the MS group (Table 3).

Immunohistochemical Expression Of Ct-1 In Cardiac Tissues

Weak cytoplasmic CT-1 expression was detected in the heart of both control (Fig. 7E) and NAC (Fig. 7F) groups. Significantly higher CT-1 expression was observed in the MS group (Fig. 7G) as compared to the control group. In the MS + NAC group (Fig. 7H), CT-1 expression was significantly lower compared to that of the MS group. while, its expression was significantly higher compared to the control group (Table 3).

Correlation Results

Significant positive correlations between cardiac CT-1 expression and aortic CT-1 expression and fasting blood glucose level in the MS group were revealed. Also, in the MS + NAC group, significant positive correlations between cardiac CT-1 expression and aortic CT-1 expression and basal glycemia were observed (Table 4).

Table 4
Pearson's correlation coefficient between cardiotrophin-1 expression levels in the heart and the aorta and physiological variables and lipid profile parameters.

		MS	MS + NAC
Basal glycemia	Cardiac CT-1	r = 0.78	r = 0.88
	Aortic CT-1	*P < 0.05**	*P* < 0.01**
		r = 0.77	r = 0.87
		P < 0.05*	*P* < 0.01**
Final SBP	Cardiac CT-1	r = 0.98	r = 0.88
	Aortic CT-1	*P < 0.001***	*P < 0.01***
		r = 0.76	r = 0.83
		*p < 0.05**	*P < 0.05**
Final DBP	Cardiac CT-1	r = 0.88	r = 0.90
	Aortic CT-1	*P < 0.01***	*P < 0.01***
		r = 0.72	r = 0.82
		*P < 0.05**	*P < 0.05**
Total cholesterol	Cardiac CT-1	r = 0.85	r = 0.90
	Aortic CT-1	*P < 0.01***	*P < 0.01***
		r = 0.89	r = 0.86
		P < 0.01 **	*P < 0.01***
Triglycerides	Cardiac CT-1	r = 0.73	r = 0.71
	Aortic CT-1	P < 0.05*	*P < 0.05**
		r = 0.82	r = 0.67
		*P < 0.05**	*p* = 0.0670(ns)

P value with an asterisk is considered significant.

*P < 0.5.

**P < 0.01.

***P < 0.001.

		MS	MS + NAC
LDL-C	Cardiac CT-1	r = 0.80	r = 0.83
	Aortic CT-1	P < 0.05*	*P < 0.05**
		r = 0.78	r = 0.81
		*P < 0.05**	*P < 0.05**
HDL-C	Cardiac CT-1	r= -0.77	r= -0.78
	Aortic CT-1	P < 0.05*	P < 0.05*
		r= -0.84	r= -0.75
		*P < 0.01***	*P < 0.05 **
P value with an asterisk is considered significant.			
*P < 0.5.			
**P < 0.01.			
***P < 0.001.			

Significant positive correlations between cardiac CT-1 and aortic CT-1 and final SBP measured in the fructose fed rats were reported. Also, cardiac CT-1 level and aortic CT-1showed significant positive correlations with the values of the final SBP in MS + NAC group. In addition, significant positive correlations between cardiac CT-1 and aortic CT-1 and final DBP were demonstrated in MS group. Moreover, in the MS + NAC animals model, significant positive correlations co-existed between cardiac CT-1 and aortic CT-1 and final DBP (Table 4).

In fructose induced MS animals group, significant positive correlations were found between cardiac CT-1 and parameters of lipid profile, namely triglycerides, total cholesterol, and LDL-C. Significant positive correlations, also, existed between aortic CT-1 and those parameters. In addition, in MS + NAC animals group, significant positive correlations were found between cardiac CT-1 and triglycerides, total cholesterol and LDL-C levels. Furthermore, aortic CT-1 was significantly positively correlated to both total cholesterol and LDL-C levels (Table 4).

On the other hand, in the MS induced animals group, cardiac CT-1 and aortic CT-1 showed significant negative correlations to HDL-C levels. In the MS + NAC group, significant negative correlation between cardiac CT-1 and aortic CT-1 and the HDL-C levels also demonstrated (Table 4).

Discussion

In this study, male Wistar rats fed with 20% fructose drinking water for 12 weeks developed MS animal model escorted with increment of body weight and body weight gain as eminent features. Several studies

have been reported an association between chronic fructose consumption and the development of obesity and MS [12, 18]. This could be ascribed to high calorie intake and boosted lipogenesis observed in chronic fructose consumption conditions [12, 19].

In our observations, co-administration of NAC resulted in a remarkable reduction of final body weight and body weight gain. In an in vitro study, NAC treatment to cultured adipocytes repressed lipid accumulation and ROS production [20]. Also, in experimental models of diet induced obesity, NAC supplementation exhibited weight reduction effect [21, 22]. Ma et al. [21] observed that NAC administration prevent lipid accumulation in brown adipose tissue which has a prominent role in thermogenesis and in mobilizing lipids utilization. Also, in their study, they revealed augmentation of thermogenic genes expression in NAC treated animals suggesting that NAC treatment may enhance energy expenditure. Furthermore, Shen et al. [22] attributed the reduction of body weight with NAC treatment to a vicious circle of suppressed OS, and increased motor activity, which aids to reduce body fat and weight.

This model also, showed IR as evidenced by hyperglycemia, hyperinsulinemia and HOMA-IR index with concomitant reduction in QUICKI index. In addition, OGTT and ITT emphasized our previous findings. The IR could be attributed to reduced adiponectin expression observed in MS [23]. Lihn et al. [24] reported that diminished expression of adiponectin has been associated with IR in animal studies indicating a role for hypoadiponectinaemia in relation to IR. Also, it has been demonstrated that hidden inflammation and adipocyte hypertrophy, lessened regenerative potential of fat progenitor cells, and impaired renewal of fat depots could be mechanisms of IR [25].

In our study, we observed an amendment of glycemic control and IR with NAC supplementation. Accumulating experimental evidence indicated that NAC promoted adiponectin gene expression, resulting in reduced hyperglycemia and hyperinsulinemia, and amelioration of IR [21, 26]. The principal mechanisms by which adiponectin improve insulin sensitivity seems to be through augmented fatty acid oxidation and suppression of hepatic glucose production [24]. Also, NAC could ameliorate IR through down-regulation of intracellular ROS and direct free radical scavenger actions [27].

In the present investigation, chronic fructose consumption encouraged OS as evidenced by increased MDA levels and decreased TAC levels. It has been long established that MS and IR have been linked to OS. Both clinical and experimental evidences displayed the relation of MS and obesity with boosting of OS [4, 28]. Also, recently Bilginoglu [29] reported an increase serum, cardiac and aortic tissues OS markers with a decrement of antioxidants levels in rats with MS. Heightening of OS in MS could be due to improper rise of free fatty acids which trigger ROS production [30]. Also, fat buildup lead to lowering of anti-oxidative enzymes which elicit ROS formation [31]. In addition, repression of P53, which is a protector genome, reported in MS. This reduces the expression of anti-oxidant enzymes; glutathione peroxidase and superoxide dismutase consequently triggers cellular ROS production, leading to tissue oxidative injury [32]. Moreover, it was found that most of diseases associated with MS as IR, hypertension, obesity, dyslipidemia might impede mitochondrial homeostasis and cause mitochondrial OS [33].

The present study confirmed the effectiveness of NAC in opposing free radical mediated oxidative insult produced by chronic fructose consumption in MS induced rats. This was evident from the reduction of MDA and the increment of TAC levels in animals co-administered NAC and fructose. NAC has been reported to scavenge free radicals, replenish reduced glutathione and inhibit its depletion, and block lipid peroxidation [34]. It can also reinstate the pro-oxidant/antioxidant imbalance via its metal-chelation activity [35]. Furthermore, NAC may be a salutary candidate to handle the mitochondrial alteration and OS. It has been reported that NAC supplementation resulted in up-regulation of mitochondrial silent information regulator 3 protein which lessens mitochondrial injury and maintain its homeostasis [36].

Hypertension is a main character of MS affecting about up to 85% of MS patients [37]. In the current investigation, both SBP and DBP are increased with chronic fructose consumption. As described in several studies, chronic fructose administration induced an early rise in blood pressure [38, 39]. This could be attributed to repression of NO bioavailability observed in IR which in turn, resulted in endothelial dysfunction and impairment of NO dependent vasodilatation [40]. Also, boosting of renal expression of renin with consequent activation of angiotensin II and aldosterone formation reported in fructose fed animals [41]. In addition, the incorporated feedbacks from afferent nerves, ROS and increased metabolic hormones such as leptin work centrally to encourage sympathetic outputs and further increase blood pressure. Moreover, amendment in sodium transporter expression and activity throughout the kidney promote plasma volume expansion which is responsible, at least in part, for the noticed hypertension [42].

Also, in our results, concurrent treatment with NAC was reported to lower elevated arterial blood pressure. This effect of NAC may be mediated by its ability to restore NO bioactivity which in turn, aids in normalization of the arterial blood pressure [43]. In addition, clinical evidence has suggested the ability of NAC to suppress the sympathetic stimulation [44]. Hence, help in blood pressure restoration in MS animals. Furthermore, NAC has been proven to restrain renin expression in the renal tissue [45]. Finally, according to Krause et al. [46], NAC by its antioxidant activity and glutathione supply could improve hypertension in cases of increased OS associated with elevated blood pressure.

In our observations, dyslipidemia with increased atherogenic index was reported in fructose induced MS animals. The results herein were in resemblance with those obtained previously by Ghibu et al. [47] who encountered dyslipidemia in rats fed high fructose diet. It has been demonstrated that high fructose intake has a lipogenic effect via de novo lipogenesis in the liver and accumulation of lipids in liver and elevated their blood values. On the long run, this excess fat intracellularly and systematically induces OS and inflammation which in turn progress forward to IR and high basal glycemia [48]. Also, the IR developed by hypertriglycerdima leads to ongoing lipolysis with more and more fatty acids and glycerol. Then, they both enter the adipose tissue to form triglycerides surpassing to a vicious circle of more triglycerides to be formed [12]. In addition, recently in a novel polygenic rat model of MS, obesity, and diabetes, Han et al. [49] reported that gene expressions involved in lipid metabolism were dysregulated with the key proteins participating in pathogenesis of dyslipidemia and IR were up-regulated.

The co-administration of NAC revealed partial recovery of lipid profile as evidenced by normalization of total cholesterol, triglycerides, HDL-cholesterol and atherogenic index and decrement of LDL-cholesterol confirming the improvement of dyslipidemia, which is supported by previous studies [21, 50, 51]. The lipid-lowering action of NAC in our MS animal model of hyperlipidemia can be partially ascribed to the suppression of mRNA expression of lipogenic related enzymes [52]. Also, in an experimental mouse model of high-sucrose diet feeding, NAC hampered the metabolic shifting in cardiac tissue, promoting fatty acid oxidation [53]. In addition, the upkeep of the normal structure of lipoprotein receptors is pivotal for their function, enabling the cellular uptake of serum lipids from the blood. On the other hand, ROS oxidize lipoproteins and prohibit lipid intracellular uptake [50]. It is possible that the decreased serum cholesterol levels in rats fed NAC-supplementation are due to the anti-oxidative effects of NAC.

CT-1 is elevated in the myocardium and plasma of heart failure patients [54], and it has been proven to be related to hypertension, cardiac hypertrophy, and fibrosis both in patients [54–56] and experimental models [57]. Interestingly, CT-1 is up-regulated in cardiac fibroblasts and cardiomyocytes in response to metabolic, humoral, mechanical and hypoxic stress [58]. The existing data showed up-regulation of CT-1 expression in both heart and aorta in MS rats. In agreement, we demonstrated an increased cardiac interstitial fibrosis in those animals. In the thoracic aorta, increased thickness of TM with deposition of connective tissue was reported.

In line with these reports, López-Andrés et al. [57] verified that CT-1 treatment increased left ventricular volumes and induced myocardial dilatation and myocardial fibrosis meanwhile, in aorta, arterial stiffness, vascular media thickness, collagen and fibronectin content were increased by CT-1 treatment. Also, it has been proposed that CT-1 accelerates the development of atherosclerotic lesions by stimulating the inflammasome, foam cell formation and collagen-1 production in vascular smooth muscle cells [59]. In addition, in an *in-vitro* study, it was found that CT-1 induces the proteolytic potential in human aortic endothelial cells by up-regulating matrix metalloproteinase-1 expression thus, may play an important role in the pathophysiology of atherosclerosis and plaque instability [60]. On the other hand, decreased arterial stiffness, media thickness and vascular wall fibrosis were demonstrated in CT-1-null mice [61].

The increase in relative heart weight in our MS animals was reported previously by Wu et al. [62] who showed an increase in the heart weight/ body weight ratio and myocardial hypertrophy in high fructose intake mice model. Furthermore, Yan et al. [63] demonstrated that intima to media thickness ratio was increased in the thoracic aorta of MS model.

The aortic stiffness reported in MS may be ascribed to thoracic aorta perivascular adipose tissue dysfunction observed in MS that through interplay between TNFα and NADPH-oxidase 2 causing aortic stiffness [64]. Also, Martinez-Martinez [65] observed that CT-1 could up-regulate cardiac galectin-3 which, in turn, mediates the proinflammatory and profibrotic myocardial effects of CT-1.

In our experiment, NAC co-treatment resulted in down-regulation of CT-1 in the heart and aorta. Also, relative heart weight was decreased by NAC supplementation. Similar results observed by Jia et al. [66]

who reported that cardiac fibroblast proliferation, collagen I and CT-1 overexpression induced by isoprenaline stimulation were effectively abrogated by NAC treatment.

The beneficial effects of NAC against cardiac hypertrophy and aortic stiffness were reported previously [67–69]. It has been deduced that reducing OS by NAC in pressure overload may prevent electrical remodeling and ameliorate hypertrophy in epicardial myocytes [67]. Also, down-regulation of CT-1 either in vivo or in vitro was shown to be a mechanism of cardioprotection in hypertrophied heart [68]. In addition, Wu et al. [69] demonstrated that NAC treatment could prevent pyroptosis which is a cellular mechanism for the pro-atherosclerotic plaque formation in human aortic endothelial cells. Furthermore, mouse and human hypertrophic cardiomyopathy were antagonized by NAC treatment through stimulation of miR-29a expression and suppression of pro-fibrotic gene TGFβ expression and secretion [70]. Also, it has been demonstrated that NAC could inhibit the decrease in collagen I/III ratio which play a role in cardiac extracellular matrix composition and cardiac hypertrophy [71].

Our histopathological findings confirmed the biochemical results. In the present investigation, histopathological examination of the heart of the MS group revealed marked degeneration of the cardiomyocytes, interstitial fibrosis and infiltration of inflammatory cells which is in agreement with the findings observed by Putakala et al. [72] who found fibrosis, degenerative changes, neutrophil infiltration and fat deposition in chronically fructose fed animals. Also, variable degrees of collagen fibers proliferation could be detected in routinely H&E stained heart specimens of high fructose diet-induced MS rats [73]. Furthermore, inflammatory cell infiltration and mast cell activation close to the blood vessels, and degeneration of myofibrils in cardiomyoctes with intercalated discs were reported by Acikel Elmas et al. [74]. This could be attributed to mast cell infiltration of the cardiac tissue which release chemokines, pro-inflammatory cytokines, histamine and proteases in MS conditions [75]. Collagen deposition in the heart of MS models was proved to be due to fructose intake which in turn caused release of ROS with subsequent promoting inflammasome followed by fibrosis [76]. NAC co-administration in our study resulted in remarkable improvement of the histopathological appearance of the cardiac tissues. This could be ascribed to down-regulation of OS which may directly or indirectly improve cardiac pathology as evidenced by our results.

The present data showed a robust positive correlation between both cardiac and aortic CT-1 expression and basal glycemia, SBP, DBP, total cholesterol, triglycerides and LDL-cholesterol and a negative correlation with HDL- cholesterol in MS and MS + NAC groups.

A reduction in serum CT-1 levels after weight-loss timetable and a strong association of decreased CT-1 with lowering of cholesterol levels were demonstrated previously [77]. In this study, CT-1 was suggested to be an indicator for the diagnosis of MS in overweight/obese children population. In a study done by Gkaliagkousi et al. [78], CT-1 levels was positively correlated with blood pressure and the indices of arterial stiffness. In another study, positive correlation between plasma CT-1 and basal glycemia, SBP and DBP were reported [79]. Also, they observed a positive association between CT-1 and arterial damage (increase intima-media thickness). In addition, in obese children, CT-1 transcript levels were reduced after

lifestyle interference [80]. Furthermore, Anik Ilhan et al. [81] demonstrated that CT-1 levels were found to be positively correlated with DBP and triglyceride levels in MS women with polycystic ovaries.

Increased expression of CT-1 in both heart and aorta are strongly related to the intensity of several parameters associated with cardiometabolic risk factors as observed in our correlation study. These observations suggested the potential involvement of CT-1 in cardiovascular injury and diseases.

Conclusion

In conclusion, NAC displayed hopeful therapeutic values in alleviation of hyperglycemia, IR, hypertension and OS and prevention of dyslipidemic profile in high-fructose intake condition. NAC had also effects mitigating aortic and myocardial degeneration and fibrosis associated with chronic fructose induced MS as evidenced by down-regulation of CT-1 expression.

Abbreviations

ANOVA
analysis of variance; AUC:area under curve; CT-1:Cardiotrophin-1; DBP:diastolic blood pressure; FDW:fructose drinking water; HDL-C:high density lipoprotein-cholesterol; H&E:Haematoxylin and Eosin; HOMA-IR:homeostasis model assessment of insulin resistance; IR:insulin resistance; ITT:insulin tolerance test; LDL-C:low density lipoprotein-cholesterol; MDA:malondialdehyde; MS:metabolic syndrome; MT:Masson's Trichome; NAC:N-acetyl cysteine; OGTT:oral glucose tolerance test; OS:oxidative stress; QUICKI:quantitative insulin-sensitivity check index; ROS:reactive oxygen species; SBP:systolic blood pressure; TAC:total antioxidants capacity; TM:tunica media.

Declarations

Ethics approval and consent to participate

All animal procedures were approved by Animal Ethics Committee of Assiut University (Institutional review board (IRB) approval NO: 17100802).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors conceived and planned the experiments. A.S.A. and M.B.T. carried out the experiment and statistical analysis. A.S.A. wrote the manuscript with support from E. A. A.. A.M.A. performed the histopathological and immunohistochemistry part of the study. All authors discussed the results and commented on the manuscript.

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References

1. Park DY, Ahn YT, Huh CS, McGregor RA, Choi MS. Dual probiotic strains suppress high fructose-induced metabolic syndrome. World J Gastroenterol. 2013;19(2):274–83.
2. Malik VS, Popkin BM, Bray GA, Després J-P, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Circulation. 2010;121(11):1356–64.
3. de Moura RF, Ribeiro C, de Oliveira JA, Stevanato E, de Mello MAR. Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols. British journal of nutrition. 2008;101(8):1178–84.
4. Diniz YS, Rocha KK, Souza GA, Galhardi CM, Ebaid GM, Rodrigues HG, Novelli Filho JLV, Cicogna AC, Novelli EL. Effects of N-acetylcysteine on sucrose-rich diet-induced hyperglycaemia, dyslipidemia and oxidative stress in rats. Eur J Pharmacol. 2006;543(1–3):151–7.
5. Central European Journal of Immunology, 2012; 37(1): p. 57–66.
6. Dodd S, Dean O, Copolov DL, Malhi GS. Berk M.N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. Expert Opin Biol Ther. 2008;8(12):1955–62.
7. Pennica D, Shaw KJ, Swanson TA, Moore MW, Shelton DL, Zioncheck KA, Rosenthal A, Taga T, Paoni NF, Wood WI. Cardiotrophin-1. Biological activities and binding to the leukemia inhibitory factor receptor/gp130 signaling complex. J Biol Chem. 1995;270(18):10915–22.
8. Asrih M, Mach F, Quercioli A, Dallegri F, Montecucco F.Update on the pathophysiological activities of the cardiac molecule cardiotrophin-1 in obesity. Mediators Inflamm, 2013; 2013: p. 370715.

9. Malavazos AE, Ermetici F, Morricone L, Delnevo A, Coman C, Ambrosi B, Corsi MM. Association of increased plasma cardiotrophin-1 with left ventricular mass indexes in normotensive morbid obesity. Hypertension. 2008;51(2):e8.
10. Hung H-C, Lu F-H, Ou H-Y, Wu H-T, Wu J-S, Yang Y-C, Chang C-J. .Increased cardiotrophin-1 in subjects with impaired glucose tolerance and newly diagnosed diabetes. Int J Cardiol. 2013;169(3):e33–4.
11. Ali MH, Messiha BA, Abdel-Latif HA. Protective effect of ursodeoxycholic acid, resveratrol, and N-acetylcysteine on nonalcoholic fatty liver disease in rats. Pharm Biol. 2016;54(7):1198–208.
12. Mamikutty N, Thent ZC, Sapri SR, Sahruddin NN, Mohd Yusof MR, Haji Suhaimi F.The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. Biomed Res Int, 2014; 2014: p. 263897.
13. Zayed EA, AinShoka AA, El Shazly KA. Abd El Latif HA.Improvement of insulin resistance via increase of GLUT4 and PPARgamma in metabolic syndrome-induced rats treated with omega-3 fatty acid or l-carnitine. J Biochem Mol Toxicol. 2018;32(11):e22218.
14. Ouyang X, Li S, Tan Y, Lin L, Yin J, Chen JDZ. Intestinal electrical stimulation attenuates hyperglycemia and prevents loss of pancreatic beta cells in type 2 diabetic Goto-Kakizaki rats. Nutr Diabetes. 2019;9(1):4.
15. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
16. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. The Journal of Clinical Endocrinology Metabolism. 2000;85(7):2402–10.
17. Takasaki Y. Serum lipid levels and factors affecting atherogenic index in Japanese children. J Physiol Anthropol Appl Hum Sci. 2005;24(4):511–5.
18. Jürgens H, Haass W, Castañeda TR, Schürmann A, Koebnick C, Dombrowski F, Otto B, Nawrocki AR, Scherer PE, Spranger J, Ristow M, Joost HG, Havel PJ. Tschöp MH.Consuming fructose-sweetened beverages increases body adiposity in mice. Obes Res. 2005;13(7):1146–56.
19. Gugliucci A. Formation of Fructose-Mediated Advanced Glycation End Products and Their Roles in Metabolic and Inflammatory Diseases. Adv Nutr. 2017;8(1):54–62.
20. Kadota Y, Toriuchi Y, Aki Y, Mizuno Y, Kawakami T, Nakaya T, Sato M, Suzuki S. Metallothioneins regulate the adipogenic differentiation of 3T3-L1 cells via the insulin signaling pathway. PloS one. 2017;12(4):e0176070.
21. Ma Y, Gao M, Liu. D.N-acetylcysteine protects mice from high fat diet-induced metabolic disorders. Pharmaceutical research. 2016;33(8):2033–42.
22. Shen F-C, Weng S-W, Tsao C-F, Lin H-Y, Chang C-S, Lin C-Y, Lian W-S, Chuang J-H, Lin T-K, Liou C-W. Wang P-W.Early intervention of N-acetylcysteine better improves insulin resistance in diet-induced obesity mice. Free Radical Res. 2019;52(11–12):1296–310.
23. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006;444(7121):881–7.

24. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev. 2005;6(1):13–21.
25. Vorotnikov AV, Stafeev IS, Menshikov MY, Shestakova MV, Parfyonova YV. Latent Inflammation and Defect in Adipocyte Renewal as a Mechanism of Obesity-Associated Insulin Resistance. Biochemistry. 2019;84(11):1329–45.
26. Berry A, Bellisario V, Panetta P, Raggi C, Magnifico MC, Arese M, Cirulli F. Administration of the Antioxidant N-Acetyl-Cysteine in Pregnant Mice Has Long-Term Positive Effects on Metabolic and Behavioral Endpoints of Male and Female Offspring Prenatally Exposed to a High-Fat Diet. Front Behav Neurosci. 2018;12:48.
27. Keshk WA, Ibrahim MA, Shalaby SM, Zalat ZA, Elseady WS. Redox status, inflammation, necroptosis and inflammasome as indispensable contributors to high fat diet (HFD)-induced neurodegeneration; Effect of N-acetylcysteine (NAC). Arch Biochem Biophys. 2019;680:108227.
28. Urakawa H, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka K, Maruyama N, Kitagawa N, Tanaka T, Hori Y. Oxidative stress is associated with adiposity and insulin resistance in men. The Journal of Clinical Endocrinology Metabolism. 2003;88(10):4673–6.
29. Bilginoglu A. Cardiovascular protective effect of pioglitazone on oxidative stress in rats with metabolic syndrome. J Chin Med Assoc. 2019;82(6):452–6.
30. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C–dependent activation of NAD (P) H oxidase in cultured vascular cells. Diabetes. 2000;49(11):1939–45.
31. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M. Shimomura I.Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Investig. 2017;114(12):1752–61.
32. Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant function of the p53 tumor suppressor. Nature medicine. 2005;11(12):1306.
33. Palmeira CM, Moreno AJ, Mitochondrial bioenergetics. 2018: Springer.
34. Samuni Y, Goldstein S, Dean OM, Berk M.The chemistry and biological activities of N-acetylcysteine. Biochimica et Biophysica Acta (BBA)-General Subjects, 2013; 1830(8): p. 4117–4129.
35. Giampreti A, Lonati D, Ragghianti B, Ronchi A, Petrolini VM, Vecchio S, Locatelli CA.N-acetyl-cysteine as effective and safe chelating agent in metal-on-metal Hip-Implanted patients: two cases. Case reports in orthopedics, 2016; 2016.
36. Peerapanyasut W, Kobroob A, Palee S, Chattipakorn N, Wongmekiat ON. -Acetylcysteine Attenuates the Increasing Severity of Distant Organ Liver Dysfunction after Acute Kidney Injury in Rats Exposed to Bisphenol A. Antioxidants. 2019;8(10):497.
37. Duvnjak L, Bulum T, Metelko Z. Hypertension and the metabolic syndrome. Diabetologia Croatica. 2008;37(4):83–9.

38. Korandji C, Zeller M, Guilland JC, Collin B, Lauzier B, Sicard P, Duvillard L, Goirand F, Moreau D, Cottin Y, Rochette L. Vergely C.Time course of asymmetric dimethylarginine (ADMA) and oxidative stress in fructose-hypertensive rats: a model related to metabolic syndrome. Atherosclerosis. 2011;214(2):310–5.
39. Cabral PD, Hong NJ, Hye Khan MA, Ortiz PA, Beierwaltes WH, Imig JD, Garvin JL. Fructose stimulates Na/H exchange activity and sensitizes the proximal tubule to angiotensin II. Hypertension. 2014;63(3):e68–73.
40. Kearney MT, Duncan ER, Kahn M, Wheatcroft SB. Insulin resistance and endothelial cell dysfunction: studies in mammalian models. Exp Physiol. 2008;93(1):158–63.
41. Xu C, Lu A, Lu X, Zhang L, Fang H, Zhou L, Yang T. Activation of Renal (Pro)Renin Receptor Contributes to High Fructose-Induced Salt Sensitivity. Hypertension. 2017;69(2):339–48.
42. Komnenov D, Levanovich PE, Rossi NF.Hypertension Associated with Fructose and High Salt: Renal and Sympathetic Mechanisms. Nutrients, 2019; 11(3).
43. Xia Z, Nagareddy PR, Guo Z, Zhang W, McNeill JH. Antioxidant N-acetylcysteine restores systemic nitric oxide availability and corrects depressions in arterial blood pressure and heart rate in diabetic rats. Free Radic Res. 2006;40(2):175–84.
44. Jouett NP, Moralez G, White DW, Eubank WL, Chen S, Tian J, Smith ML, Zimmerman MC. Raven PB.N-Acetylcysteine reduces hyperacute intermittent hypoxia-induced sympathoexcitation in human subjects. Exp Physiol. 2016;101(3):387–96.
45. Thieme K, Da Silva KS, Fabre NT, Catanozi S, Monteiro MB, Santos-Bezerra DP, Costa-Pessoa JM, Oliveira-Souza M, Machado UF, Passarelli M. Correa-Giannella ML.N-Acetyl Cysteine Attenuated the Deleterious Effects of Advanced Glycation End-Products on the Kidney of Non-Diabetic Rats. Cell Physiol Biochem. 2016;40(3–4):608–20.
46. Krause BJ, Casanello P, Dias AC, Arias P, Velarde V, Arenas GA, Preite MD, Iturriaga R. Chronic Intermittent Hypoxia-Induced Vascular Dysfunction in Rats is Reverted by N-Acetylcysteine Supplementation and Arginase Inhibition. Front Physiol. 2018;9:901.
47. Ghibu S, Craciun CE, Rusu R, Morgovan C, Mogosan C, Rochette L, Gal AF, Dronca M.Impact of Alpha-Lipoic Acid Chronic Discontinuous Treatment in Cardiometabolic Disorders and Oxidative Stress Induced by Fructose Intake in Rats. Antioxidants (Basel), 2019; 8(12).
48. Park D-Y, Ahn Y-T, Huh C-S, McGregor RA, Choi M-S. .Dual probiotic strains suppress high fructose-induced metabolic syndrome. World Journal of Gastroenterology: WJG. 2013;19(2):274.
49. Han L, Bittner S, Dong D, Cortez Y, Bittner A, Chan J, Umar M, Shen WJ, Peterson RG, Kraemer FB. Azhar S.Molecular changes in hepatic metabolism in ZDSD rats-A new polygenic rodent model of obesity, metabolic syndrome, and diabetes. Biochim Biophys Acta Mol Basis Dis, 2020: p. 165688.
50. Korou LM, Agrogiannis G, Koros C, Kitraki E, Vlachos IS, Tzanetakou I, Karatzas T, Pergialiotis V, Dimitroulis D, Perrea DN. Impact of N-acetylcysteine and sesame oil on lipid metabolism and hypothalamic-pituitary-adrenal axis homeostasis in middle-aged hypercholesterolemic mice. Sci Rep. 2014;4:6806.

51. Kaga AK, Barbanera PO, do Carmo NOL, Rosa LRO, Fernandes AAH.Effect of N-Acetylcysteine on Dyslipidemia and Carbohydrate Metabolism in STZ-Induced Diabetic Rats. Int J Vasc Med, 2018; 2018: p. 6428630.
52. Lin C-c. Yin M-c.Effects of cysteine-containing compounds on biosynthesis of triacylglycerol and cholesterol and anti-oxidative protection in liver from mice consuming a high-fat diet. Br J Nutr. 2008;99(1):37–43.
53. Novelli ELB, Santos PP, Assalin HB, Souza G, Rocha K, Ebaid GX, Seiva FRF, Mani F. Fernandes AA.N-acetylcysteine in high-sucrose diet-induced obesity: energy expenditure and metabolic shifting for cardiac health. Pharmacological research. 2009;59(1):74–9.
54. López B, González A, Querejeta R, Larman M, Rábago G, Díez J. Association of cardiotrophin-1 with myocardial fibrosis in hypertensive patients with heart failure. Hypertension. 2014;63(3):483–9.
55. López B, González A, Querejeta R, Barba J, Díez J. Association of plasma cardiotrophin-1 with stage C heart failure in hypertensive patients: potential diagnostic implications. Journal of hypertension. 2009;27(2):418–24.
56. González A, López B, Ravassa S, Beaumont J, Zudaire A, Gallego I, Brugnolaro C. Díez J.Cardiotrophin-1 in hypertensive heart disease. Endocrine. 2012;42(1):9–17.
57. López-Andrés N, Rousseau A, Akhtar R, Calvier L, Iñigo C, Labat C, Zhao X, Cruickshank K, Díez J. Zannad F.Cardiotrophin 1 is involved in cardiac, vascular, and renal fibrosis and dysfunction. Hypertension. 2012;60(2):563–73.
58. Hogas S, Bilha SC, Branisteanu D, Hogas M, Gaipov A, Kanbay M, Covic A. Potential novel biomarkers of cardiovascular dysfunction and disease: cardiotrophin-1, adipokines and galectin-3. Archives of medical science: AMS. 2017;13(4):897.
59. Konii H, Sato K, Kikuchi S, Okiyama H, Watanabe R, Hasegawa A, Yamamoto K, Itoh F, Hirano T. Watanabe T.Stimulatory effects of cardiotrophin 1 on atherosclerosis. Hypertension. 2013;62(5):942–50.
60. Tokito A, Jougasaki M, Ichiki T, Hamasaki S. Cardiotrophin-1 induces matrix metalloproteinase-1 in human aortic endothelial cells. PLoS One. 2013;8(7):e68801.
61. Lopez-Andres N, Calvier L, Labat C, Fay R, Diez J, Benetos A, Zannad F, Lacolley P. Rossignol P.Absence of cardiotrophin 1 is associated with decreased age-dependent arterial stiffness and increased longevity in mice. Hypertension. 2013;61(1):120–9.
62. Wu X, Pan B, Wang Y, Liu L, Huang X, Tian J. The protective role of low-concentration alcohol in high-fructose induced adverse cardiovascular events in mice. Biochem Biophys Res Commun. 2018;495(1):1403–10.
63. Yan LJ, Wang SB, Wang XQ, Cao XM. [Protective effect and mechanism of curcumin on aorta in rats with metabolic syndrome]. Zhongguo Zhong Yao Za Zhi. 2019;44(21):4685–90.
64. DeVallance E, Branyan KW, Lemaster K, Olfert IM, Smith DM, Pistilli EE, Frisbee JC, Chantler PD. Aortic dysfunction in metabolic syndrome mediated by perivascular adipose tissue TNFalpha- and NOX2-dependent pathway. Exp Physiol. 2018;103(4):590–603.

65. Martinez-Martinez E, Brugnolaro C, Ibarrola J, Ravassa S, Buonafine M, Lopez B, Fernandez-Celis A, Querejeta R, Santamaria E, Fernandez-Irigoyen J, Rabago G, Moreno MU, Jaisser F, Diez J, Gonzalez A. Lopez-Andres N.CT-1 (Cardiotrophin-1)-Gal-3 (Galectin-3) Axis in Cardiac Fibrosis and Inflammation. Hypertension. 2019;73(3):602–11.
66. Jia G, Leng B, Wang H, Dai H. Inhibition of cardiotrophin1 overexpression is involved in the antifibrotic effect of Astrogaloside IV. Mol Med Rep. 2017;16(6):8365–70.
67. Foltz WU, Wagner M, Rudakova E. Volk T.N-acetylcysteine prevents electrical remodeling and attenuates cellular hypertrophy in epicardial myocytes of rats with ascending aortic stenosis. Basic Res Cardiol. 2012;107(5):290.
68. Al-Mazroua HA, Al-Rasheed NM, Korashy HM. Downregulation of the cardiotrophin-1 gene expression by valsartan and spironolactone in hypertrophied heart rats in vivo and rat cardiomyocyte H9c2 cell line in vitro: a novel mechanism of cardioprotection. J Cardiovasc Pharmacol. 2013;61(4):337–44.
69. Wu X, Zhang H, Qi W, Zhang Y, Li J, Li Z, Lin Y, Bai X, Liu X, Chen X, Yang H, Xu C, Zhang Y. Yang B.Nicotine promotes atherosclerosis via ROS-NLRP3-mediated endothelial cell pyroptosis. Cell Death Dis. 2018;9(2):171.
70. Liu Y, Afzal J, Vakrou S, Greenland GV, Talbot CC Jr, Hebl VB, Guan Y, Karmali R, Tardiff JC, Leinwand LA, Olgin JE, Das S, Fukunaga R. Abraham MR.Differences in microRNA-29 and Pro-fibrotic Gene Expression in Mouse and Human Hypertrophic Cardiomyopathy. Front Cardiovasc Med. 2019;6:170.
71. Ninh VK, El Hajj EC, Ronis MJ. Gardner JD.N-Acetylcysteine prevents the decreases in cardiac collagen I/III ratio and systolic function in neonatal mice with prenatal alcohol exposure. Toxicol Lett. 2019;315:87–95.
72. Putakala M, Gujjala S, Nukala S, Bongu SBR, Chintakunta N, Desireddy S. Cardioprotective effect of Phyllanthus amarus against high fructose diet induced myocardial and aortic stress in rat model. Biomed Pharmacother. 2017;95:1359–68.
73. Mostafa-Hedeab G, Shahataa M, Fouaad Ali E, Sabry D, El-Nahass ELS, Hassan M, Mahmoud FAllopurinol. Ameliorates High Fructose Diet-Induced Metabolic Syndrome via up-regulation of Adiponectin Receptors and Heme oxygenase-1 Expressions in Rats. Biomedical Pharmacology Journal. 2017;10(4):1685–94.
74. Acikel Elmas M, Cakici SE, Dur IR, Kozluca I, Arinc M, Binbuga B, Bingol Ozakpinar O, Kolgazi M, Sener G. Ercan F.Protective effects of exercise on heart and aorta in high-fat diet-induced obese rats. Tissue Cell. 2019;57:57–65.
75. Theoharides TC, Sismanopoulos N, Delivanis D-A, Zhang B, Hatziagelaki EE, Kalogeromitros D. Mast cells squeeze the heart and stretch the gird: their role in atherosclerosis and obesity. Trends Pharmacol Sci. 2011;32(9):534–42.
76. Kang L-L, Zhang D-M, Ma C-H, Zhang J-H, Jia K-K, Liu J-H, Wang R, Kong L-D. .Cinnamaldehyde and allopurinol reduce fructose-induced cardiac inflammation and fibrosis by attenuating CD36-mediated TLR4/6-IRAK4/1 signaling to suppress NLRP3 inflammasome activation. Scientific reports. 2016;6(1):1–18.

77. Rendo-Urteaga T, Garcia-Calzon S, Martinez-Anso E, Chueca M, Oyarzabal M, Azcona-Sanjulian MC, Bustos M, Moreno-Aliaga MJ, Martinez JA. Marti A.Decreased cardiotrophin-1 levels are associated with a lower risk of developing the metabolic syndrome in overweight/obese children after a weight loss program. Metabolism. 2013;62(10):1429–36.
78. Gkaliagkousi E, Gavriilaki E, Nikolaidou B, Chatzopoulou F, Anyfanti P, Triantafyllou A, Petidis K, Zamboulis C, Douma S. Association between cardiotrophin 1 levels and central blood pressure in untreated patients with essential hypertension. Am J Hypertens. 2014;27(5):651–5.
79. Gamella-Pozuelo L, Fuentes-Calvo I, Gomez-Marcos MA, Recio-Rodriguez JI, Agudo-Conde C, Fernandez-Martin JL, Cannata-Andia JB, Lopez-Novoa JM, Garcia-Ortiz L. Martinez-Salgado C.Plasma Cardiotrophin-1 as a Marker of Hypertension and Diabetes-Induced Target Organ Damage and Cardiovascular Risk. Medicine. 2015;94(30):e1218.
80. Marti A, Morell-Azanza L, Rendo-Urteaga T, Garcia-Calzon S, Ojeda-Rodriguez A, Martin-Calvo N, Moreno-Aliaga MJ, Martinez JA. Azcona-San Julian MC.Serum and gene expression levels of CT-1, IL-6, and TNF-alpha after a lifestyle intervention in obese children. Pediatr Diabetes. 2018;19(2):217–22.
81. Anik Ilhan G, Kanlioglu C, Arslan G, Yildizhan B, Pekin T. Cardiotrophin-1 as a new metabolic biomarker in women with PCOS. Gynecol Endocrinol. 2018;34(9):781–3.

Figures

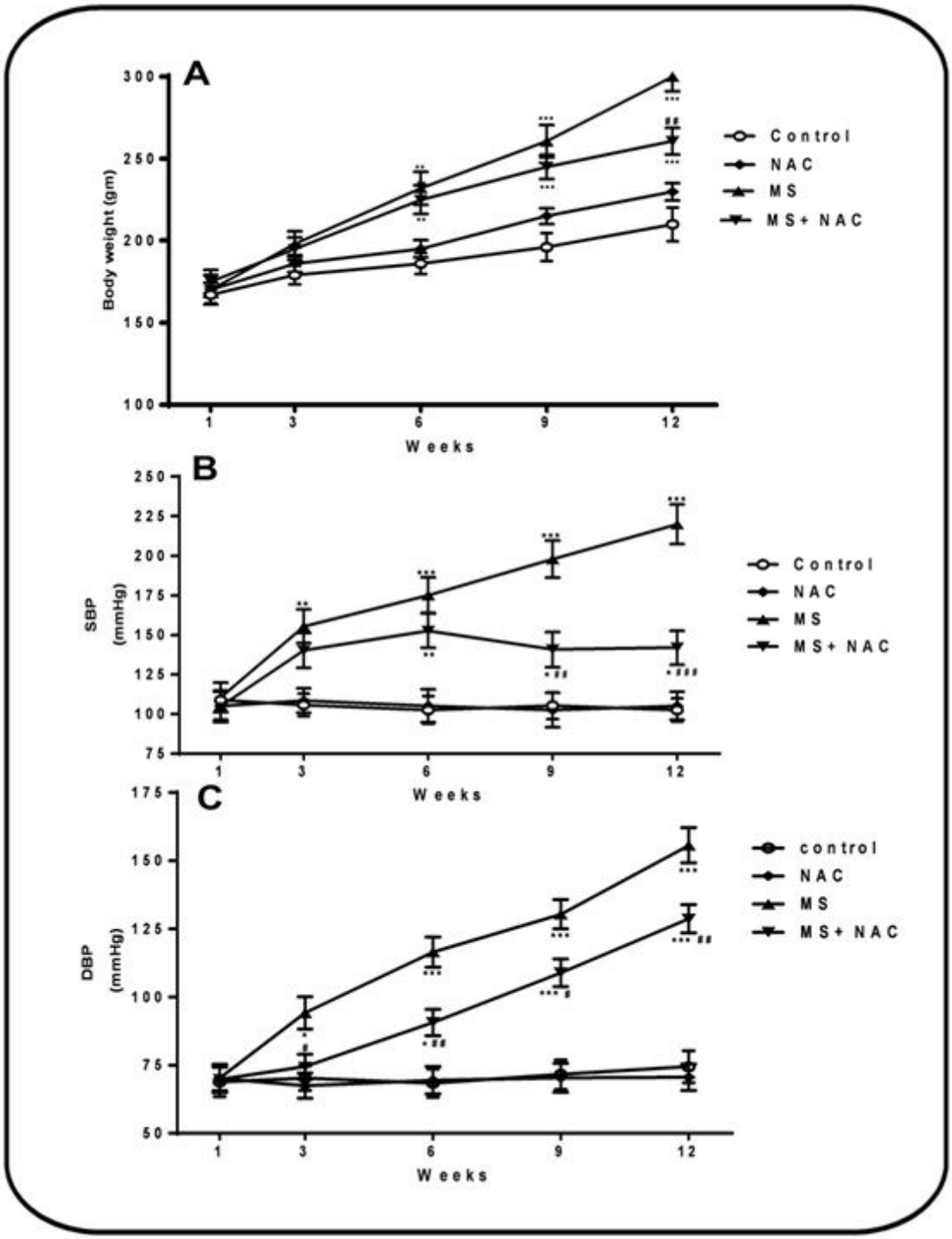

Figure 1

Changes in body weight (A), systolic blood pressure (SBP) (B) and diastolic blood pressure (DBP) (C) throughout the weeks of the experiment. Data are presented as mean ± SE (n = 8 rats in each group). Data were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. *Significantly different vs. control group (P<0.05) ** Significantly different vs. control group (P<0.01) ***Significantly different vs. control group (P<0.001). # Significantly different vs. MS group (P < 0.05). ## Significantly different vs. MS group (P < 0.01). ### Significantly different vs. MS group (P <0.001).

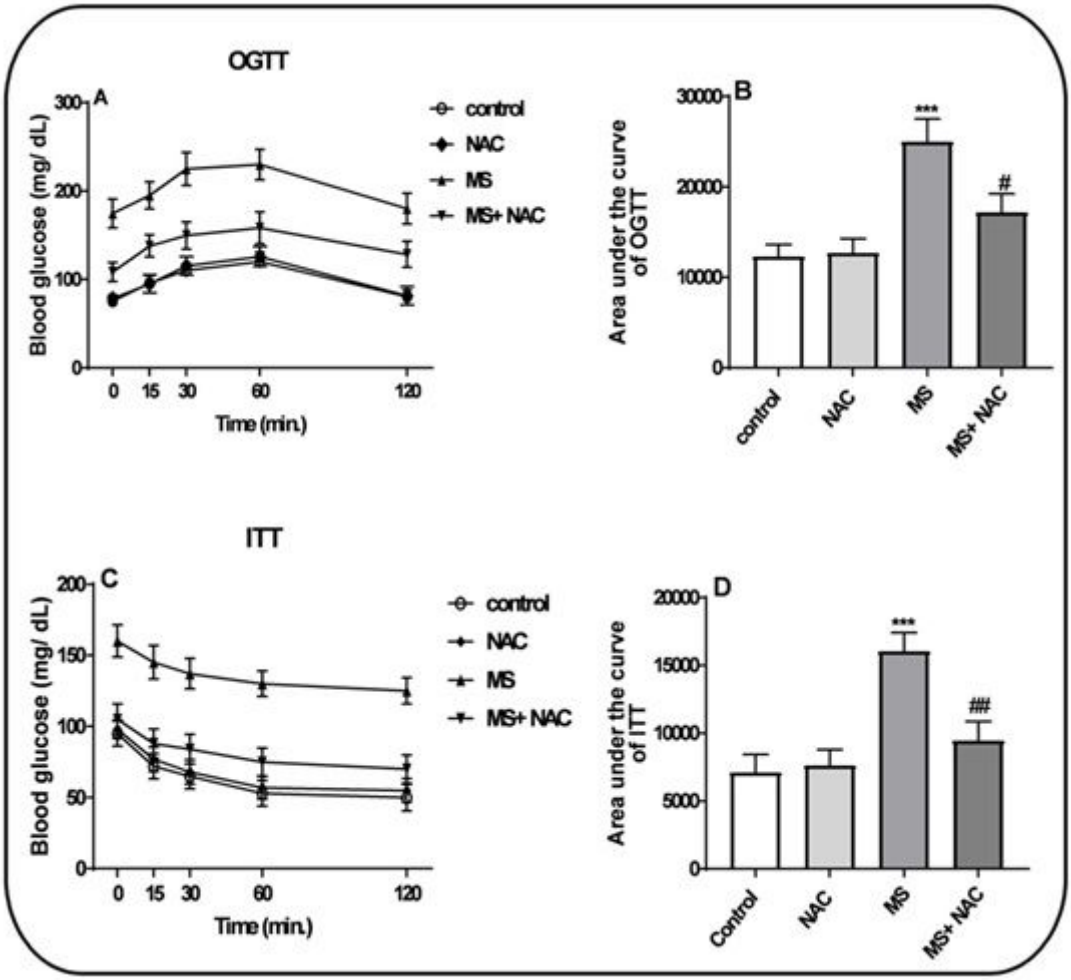

Figure 2

Oral glucose tolerance test (OGTT) (A) and insulin tolerance test (ITT) (C) and area under the curve (AUC) (B& D) for both tests. Data are presented as mean ± SE (n = 8 rats in each group).Data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. ***Significantly different vs. control group (P<0.001) # Significantly different vs. MS group (P < 0.05) ## Significantly different vs. MS group (P < 0.01)

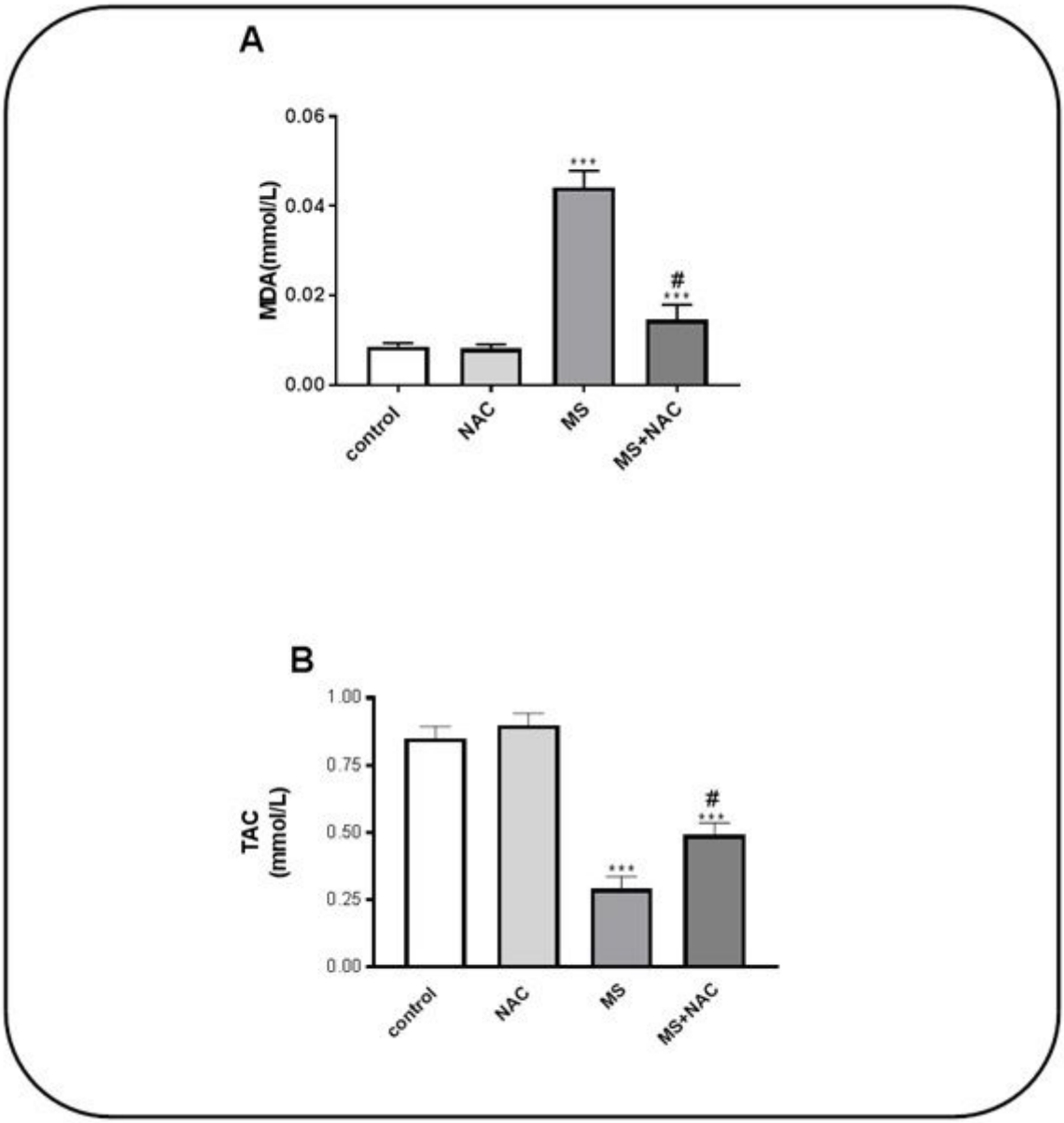

Figure 3

Oxidative stress variables of the studied groups. Malondialdehyde (MDA)(A) and total antioxidants capacity (TAC) (B). Data are presented as mean ± SE (n = 8 rats in each group). Data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. *** Significantly different vs. control group ($P < 0.001$). # Significantly different vs. MS group ($P < 0.05$).

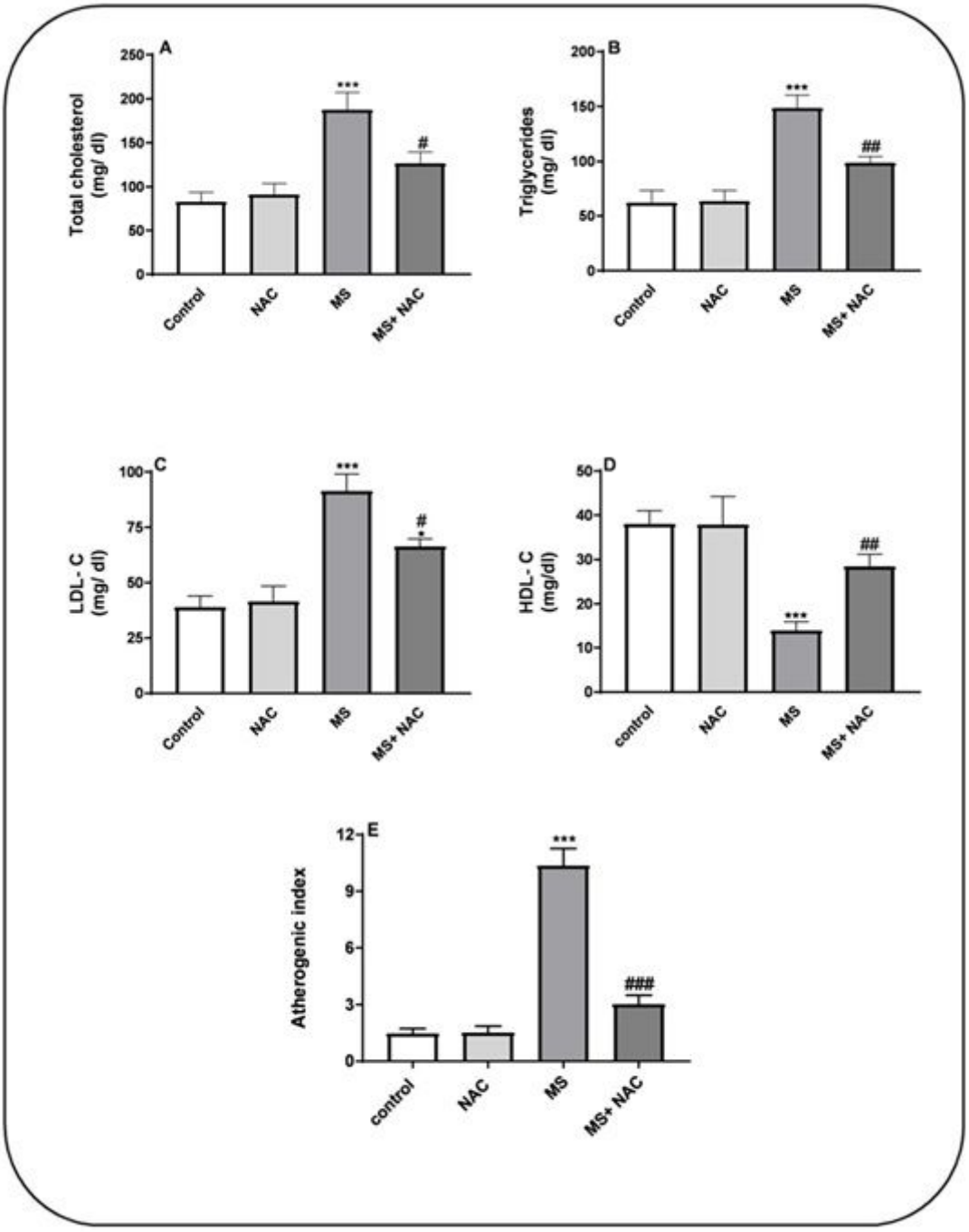

Figure 4

Serum Lipid profiles and atherogenic index of the studied groups. Data are presented as mean ± SE (n = 8 rats in each group). Data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. *Significantly different vs. control group (P<0.05) *** Significantly different vs. control group (P< 0.001) #Significantly different vs. MS group (P < 0.05) ## Significantly different vs. MS group (P < 0.01). ### Significantly different vs. MS group (P <0.001)

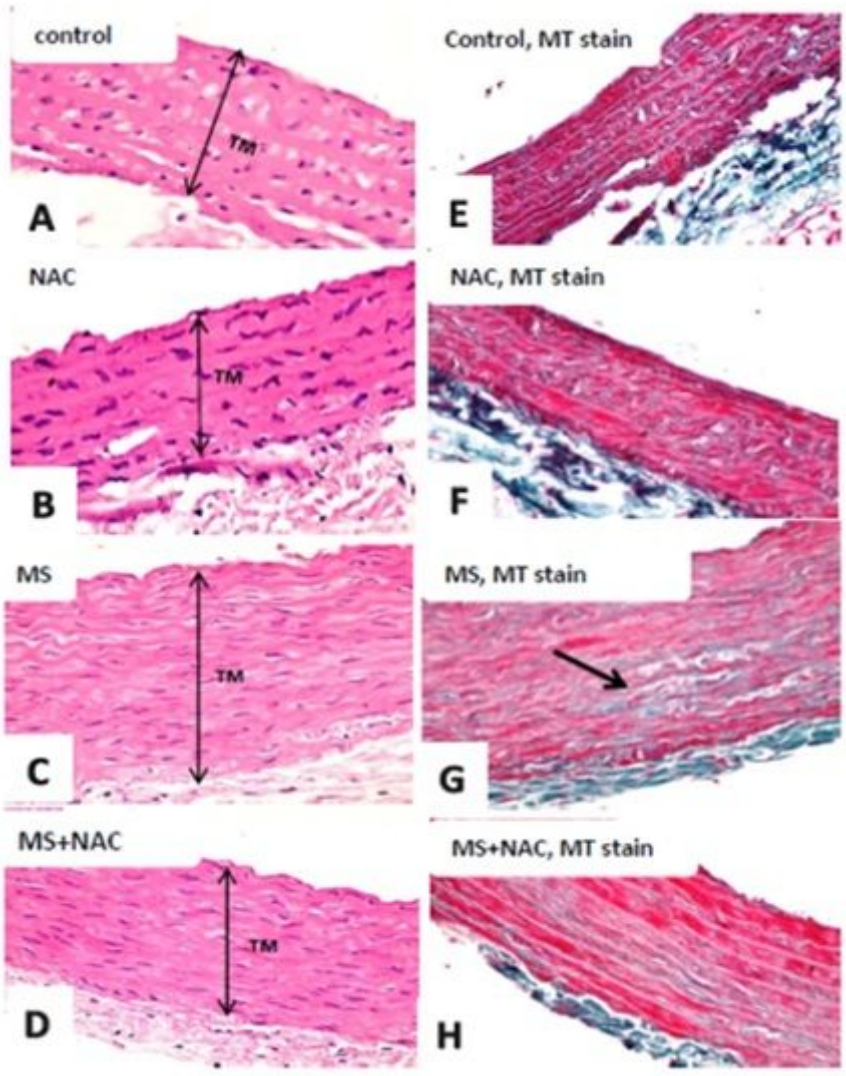

Figure 5

Histologic examination of representative sections of aortic tissues (x400). No pathologic changes in the control group (A, H&E stain) and the NAC group (B, H&E stain). Increased thickness of the tunica media (TM) (arrow) in MS group (C, H&E stain). The MS+NAC group shows decreased thickness of TM (arrow) (D, H&E stain). Masson's Trichrome (MT) stained sections show: No obvious connective tissue deposition in the TM of the aortic tissue of the control group (E x400) and the NAC group (F x400). Increased connective tissue deposition in the TM of the aorta in the MS group (arrow) (G x400). Lesser connective tissue deposition in the MS+NAC group (H x400) than in the MS group.

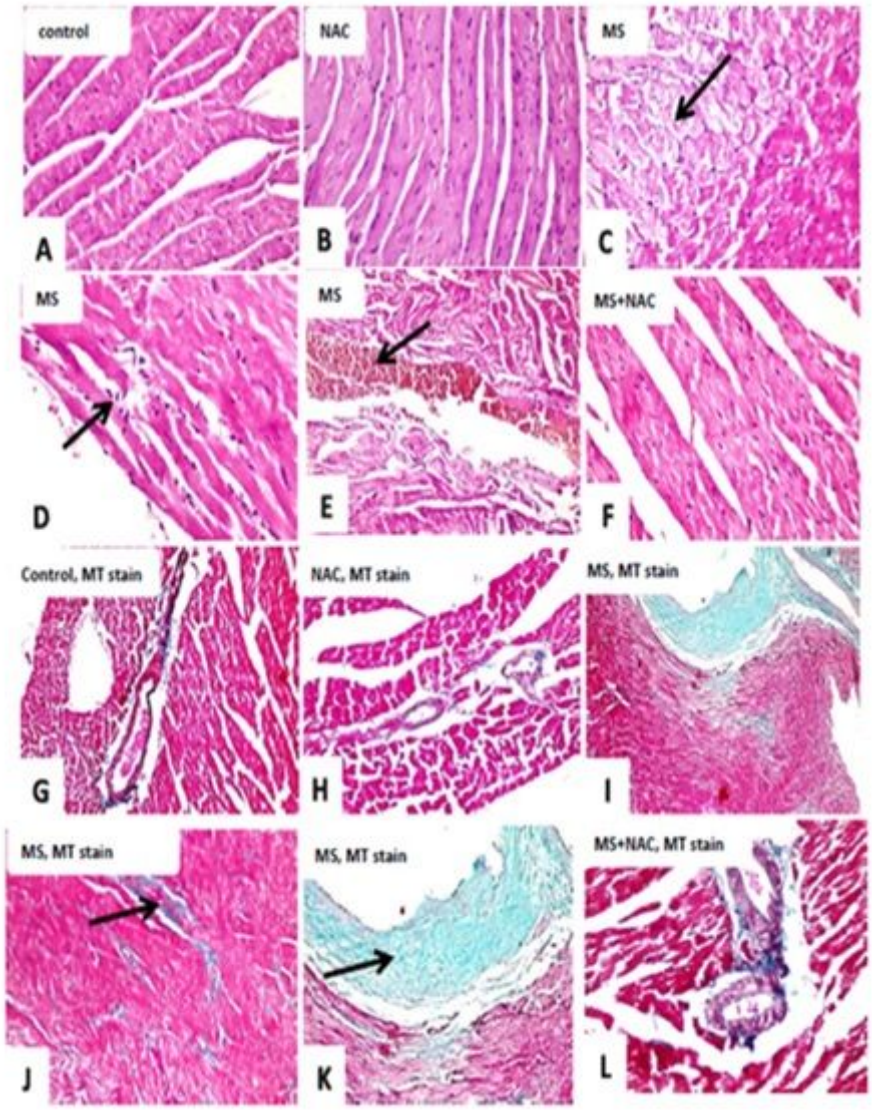

Figure 6

Histologic examination of representative sections of cardiac tissues (x400). No pathologic changes in the control group (A, H&E stain) and the NAC group (B, H&E stain). The MS group shows vacuolar degeneration of the cardiomyocytes (arrow) (C, H&E stain), mononuclear inflammatory cellular infiltrate (arrow) (D, H&E stain) and interstitial hemorrhage (arrow) (E, H&E stain). The MS+NAC group shows improvement of all these histopathologic changes (F ,H&E stain). Masson's Trichrome (MT) stain shows: Absence of interstitial fibrosis in the control group (G, x400) and the NAC group (H, x400). The MS group shows increased interstitial fibrosis and connective tissue deposition in the wall of the blood vessels (I, x200). Higher power to demonstrate increased interstitial fibrosis (arrow) (J, x400) and connective tissue deposition in the wall of the blood vessels (arrow) (K, x400) in the MS group. No interstitial fibrosis in the MS+NAC group (L, x400).

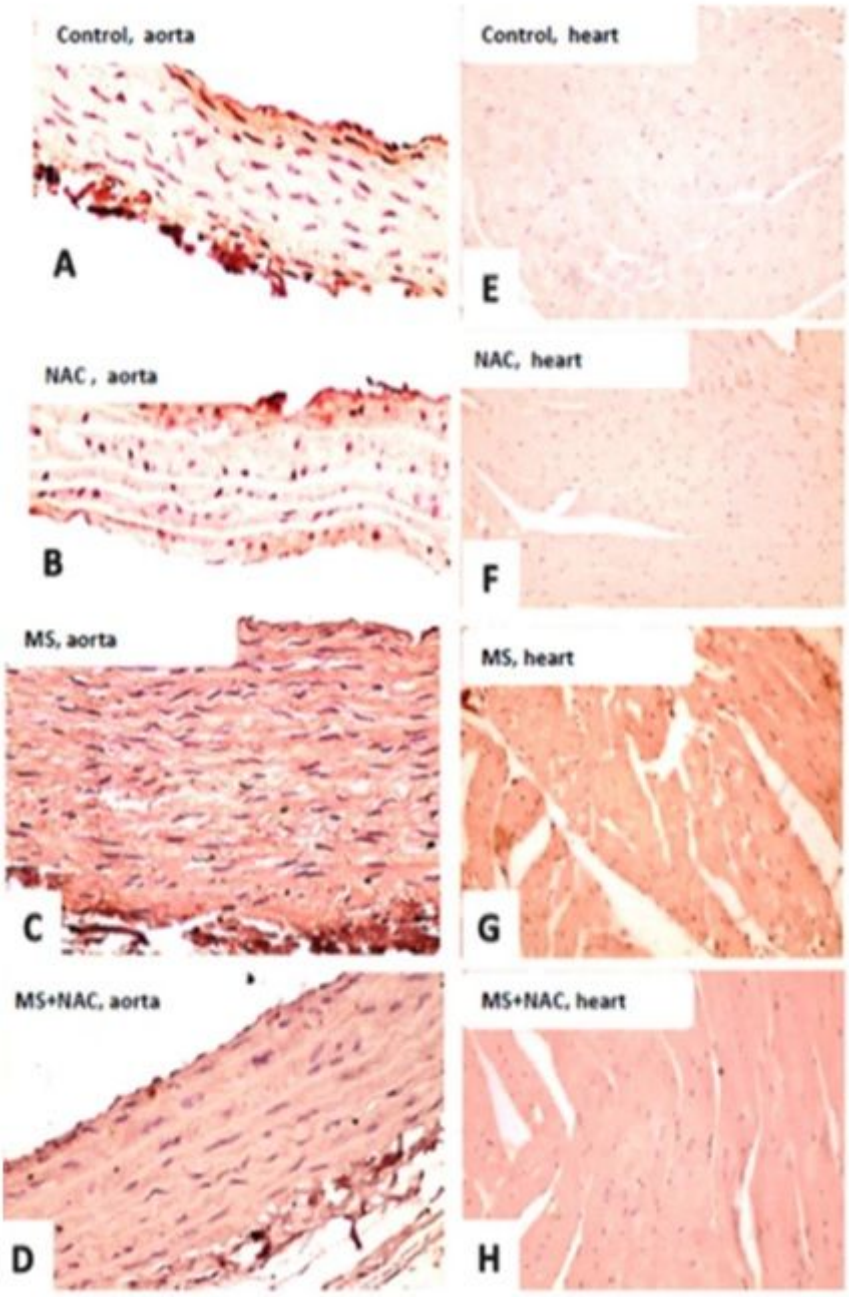

Figure 7

Immunohistochemical expression of cardiotrophin-1 (x400). Weak CT-1 expression in aorta of the control group (A) and NAC group (B). Strong CT-1 expression in aorta of the MS group (C). Moderate CT-1 expression in aorta of the MS+NAC group (D). Weak CT-1 expression in the heart of the control group (E) and NAC group (F). Strong CT-1 expression in the heart of the MS group (G). Moderate CT-1 expression in the heart of the MS+NAC group (H).