N-acetyl cysteine can blunt metabolic and cardiovascular effects via down-regulation of cardiotrophin-1 in rat model of fructose-induced metabolic syndrome

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ABSTRACT

In this study, we investigated the ability of N-acetyl cysteine (NAC) to alleviate the metabolic disorders in fructose-induced metabolic syndrome (MS) in male 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 induced MS animals for 12 weeks. Chronic fructose consumption (20% w/v) increased body weight gain, relative heart weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), insulin resistance (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 dyslipidaemia and oxidative stress. Additionally, NAC down-regulated CT-1 expression in the heart and aorta. These findings demonstrated the protective effect of NAC against aortic and myocardial degeneration and fibrosis through down-regulation of CT-1 in fructose induced MS animal model.

Introduction

Metabolic syndrome (MS) is a promptly growing global pandemic. Epidemiological research reported that the prevalence of MS was approximately one-quarter of the world’s adult population (Saklayen 2018) and was estimated to affect 25% of Middle East countries population (Ansarimoghaddam et al. 2018). MS is associated with a greater threat of numerous chronic pathologies comprising cardiovascular diseases and type 2 diabetes. It is characterised by a cluster of metabolic abnormalities including elevated fasting glucose, insulin resistance (IR), obesity, atherogenic dyslipidaemia, hypertension, and low-grade inflammation (Park et al. 2013a). One of the main contributing operators 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 (Malik et al. 2010). Animals nourished a high-fructose diet develop clinical features of MS such as IR, dyslipidaemia, and adiposity which may be helpful for evaluating possible curative interventions against MS (de Moura et al. 2008).

N-acetyl cysteine (NAC) is a pleiotropic molecule found in plants of the Allium species, especially in the onion (Diniz et al. 2006). 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) (Magdalena Radomska-Le sniewska et al. 2012). 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 (Dodd et al. 2008).

Cardiotrophin-1 (CT-1) is a protein that first 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 (Pennica et al. 1995). 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 (Asrih et al. 2013). Growing evidence showed increased circulating CT-1 levels in humans with obesity (Malavazos et al. 2008) and type 2 diabetes (Hung et al. 2013), 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 hyperlipidaemia, hyperglycaemia, IR, and oxidative stress (OS) in those rats.
Materials and methods

Animals and diets

Thirty-two healthy adult male Wistar rats with a body weight of 160–190 g were supplied by the Animal’s House Facility, Faculty of Medicine, Assiut 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 acclimatised to the housing facility for seven days with free access to standard laboratory pellet food and water prior to the experiment.

Ethical declaration

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, Ali et al. 2016).
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 (Mamikutty et al. 2014). 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).

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 (Zayed et al. 2018).

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 metre (FIA Biomed glucometer, Emsdetten, Germany).

Blood pressure measurement

Blood pressure was measured by the tail-cuff method in all groups of rats using LE5001 Non-Invasive Blood Pressure Metre with sphygmomanometer technique (Panlab Harvard Apparatus, Barcelona, Spain) at 1st, 3rd, 6th, 9th, and 12th week. Three readings were taken, then, the average reading was calculated and taken as the final reading (Mamikutty et al. 2014).

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

Oral glucose and ITTs 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) in a saline solution (0.5 U/kg) was intraperitoneal (ip) 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 area under the curve (AUC) (Ouyang et al. 2019).

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). The IR was estimated by the homeostasis model assessment of IR (HOMA-IR) (Matthews et al. 1985), and insulin sensitivity was estimated by quantitative insulin-sensitivity check index (QUICKI) (Katz et al. 2000), according to the following formulae:

\[
\text{HOMA-IR} = \frac{\text{insulin (µIU/mL)} \times \text{glucose (mmol/L)}}{22.5}
\]

\[
\text{QUICKI} = \frac{1}{\log \text{fasting insulin level (µU/mL)}} + \log \text{fasting blood glucose (mg/dL)}.
\]
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 euthanised. 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 were 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 (Zayed et al. 2018):

\[
\text{Relative heart weight} = \frac{\text{absolute heart weight (g)} 	imes 100}{\text{body weight of rat on sacrifice day (g)}}
\]

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, Cairo, Egypt) according to the manufacturer’s instructions.

Estimation of lipid profile and atherogenic 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 (Cairo, Egypt). The atherogenic index was calculated as demonstrated by Takasaki (2005), atherogenic index = (total cholesterol – HDL cholesterol)/HDL-cholesterol.

Histopathological examination

Left ventricular and thoracic aortic tissues were fixed in 10% formaldehde, then dehydrated with alcohol series and embedded in paraffin wax by routine protocols. Five micrometres 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 ×400 using a digital image analysis system (Leica Qwin 500; Leica, Cambridge, UK).

Immunohistochemistry

For immunohistochemical staining, sections were deparaffinised 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, catalogue # PA5-71926, dilution 1:100, Thermo Scientific Corporation, Fremont, CA) 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). 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 mean ± standard error of the mean. Statistical analysis of the difference between groups was carried out 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 analyse associations between quantitative variables. Fisher’s exact test was used to compare the difference in distribution frequencies among different groups. Student’s t-test and Mann–Whitney’s U test were calculated to test the mean differences in continuous variables between groups (parametric and non-parametric). Multivariate logistic regression analysis was calculated to investigate the significant factors influencing fibrosis and aortic/myocardial degeneration (odds ratio (OR), and 95% confidence interval (CI)). Significant test results were considered when p-values were < .05. All statistics were carried out using GraphPad Prism software version 7 (GraphPad, San Diego, CA) and Statistical Package for Social Sciences (IBM-SPSS/PC/VER 21, Armonk, NY).

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).

Also, MS group showed remarkable increase in final systolic blood pressure (SBP) level and final diastolic blood pressure (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 (Figure 1(A)).

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
For ITT, the insulin sensitivity was significantly higher in the MS group compared to rats administered fructose only. Comparing the AUC, there was a significant trend towards a decrease in MDA level in comparison with the MS group (Figure 3). Serum level of MDA was remarkably higher in the MS group compared to the control group. Treatment with NAC to MS induced animals resulted in significant rise in TAC level in comparison with the MS group. Meanwhile, MS animals showed an obvious decrease in TAC level compared to the control group. Treatment with NAC 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 (Figure 4).

**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 (Figure 4).

**Histopathologic examination of the aorta**

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

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

**Histopathologic examination of the cardiac tissues**

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

| Variables                  | 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***  |

SBP: systolic blood pressure; DBP: diastolic blood pressure.

Data are presented as mean ± SE (n = 8 rats in each group). Data were analysed by a one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test.

***Significantly different vs. control group (p < .001).
## Significantly different vs. MS group (p < .01).
### Significantly different vs. MS group (p < .001).
Table 2. Blood glucose homeostatic variables of the studied groups.

| Variables         | 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*** |

HOMA-IR: homeostasis model assessment of insulin resistance; QUICKI: quantitative insulin-sensitivity check index.

Data are presented as mean ± SE (n = 8 rats in each group). Data were analysed by a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test.

*Significantly different vs. control group (p < .05).
**Significantly different vs. control group (p < .01).
***Significantly different vs. control group (p < .001).
##Significantly different vs. MS group (p < .01).
###Significantly different vs. MS group (p < .001).

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 analysed by two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test.

*Significantly different vs. control group (p < .05).
**Significantly different vs. control group (p < .01).
***Significantly different vs. control group (p < .001).
##Significantly different vs. MS group (p < .01).
###Significantly different vs. MS group (p < .001).
Meanwhile, in the MS group (Figure 6(I)) increased interstitial fibrosis (Figure 6(J)) and connective tissue deposition in the wall of the blood vessels (Figure 6(K)) were observed. No interstitial fibrosis was detected in the MS + NAC group (Figure 6(L)).

**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 (Figure 7(A)) and NAC (Figure 7(B)) groups. In the MS group (Figure 7(C)), significantly higher CT-1 expression was observed as compared to the control group. Decreased CT-1 expression was observed in the MS + NAC group (Figure 7(D)) as 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 (Figure 7(E)) and NAC (Figure 7(F)) groups. Significantly higher CT-1 expression was observed in the MS group (Figure 7(G)) as compared to the control group. In the MS + NAC group (Figure 7(H)), CT-1 expression was significantly lower as compared to that of the MS group. While, its expression was significantly higher as 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 glycaemia were observed (Table 4).

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-1 showed 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 was also demonstrated (Table 4).

**Logistic regression results**

In Table 5 and Figure 8, statistically significant association between severity of fibrosis and aortic/myocardial degeneration and treatment status (p = .039) was illustrated. NAC treated rats showed lower percentages of moderate/severe fibrosis and degeneration (25%) compared with untreated MS rats (62.5%). Table 6 shows the bivariate correlates of MS severity of fibrosis and degeneration. Higher percentages of moderate/severe fibrosis and degeneration were found among untreated MS rats, with aortic and cardiac CT-1, higher HOMA-IR, LDL, MDA, TAC levels, and higher MDA/TAC ratio.

Table 7 displays the predictors of MS disease severity among the studied rats. In the final multivariate regression model, there were five predictors: MS group, aortic CT-1, HOMA-IR, MDA, and TAC. Namely, untreated MS rats were four times more liable to have moderate/severe fibrosis and aortic/myocardial degeneration (AOR = 4.02, 95% CI: 1.6–22.8, p-values = .043). Likewise, rats with increased aortic CT-1 expression were 9.6 times more likely to have moderate/severe disease grades (AOR = 9.6, 95% CI: 1.2–48.2, p-values = .037). Also, with one unit increase in the level of HOMA-IR, there was 3.2% increase in the probability of having moderate/severe disease grades (AOR = 1.032, 95% CI: 1.0–1.5, p-values = .048). Moreover, with one unit increase in the level of MDA, there was 5.6% increase in the likelihood of having moderate/severe disease grades (AOR = 1.056, 95% CI: 1.0–3.0, p-values = .045). Furthermore, with one unit increase in the level of TAC, there was 24% decrease in the likelihood of having moderate/severe disease grades (AOR = 0.74, 95% CI: 0.41–0.93, p-values = .041). Additionally, with one unit increase in MDA/TAC ratio, there was 4.2 times increase in the likelihood of having moderate/severe disease grades (AOR = 4.2, 95% CI: 1.4–8.3, p-values = .043).

**Discussion**

In this study, male Wistar rats fed with 20% FDW 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 (Jürgens et al. 2005, Mamikutty et al. 2014). This could be ascribed to high calorie intake and boosted lipogenesis observed in chronic fructose consumption conditions (Mamikutty et al. 2014, Gugliucci 2017).

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 (Kadota et al. 2017). Also, in experimental models of diet induced obesity, NAC supplementation exhibited weight reduction effect (Ma et al. 2016, Shen et al. 2018). Ma et al. (2016) observed that NAC administration prevent lipid accumulation in brown adipose tissue which has a prominent role in thermogenesis and in mobilising lipids utilisation. 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. (2018) 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 hyperglycaemia, hyperinsulinaemia and HOMA-IR index with concomitant reduction in QUICKI index. In addition, OGTT and ITT emphasised our previous findings. The IR could be attributed
to reduced adiponectin expression observed in MS (Despres et al. 2006). Lihn et al. (2005) 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 (Vorotnikov et al. 2019).

In our study, we observed an amendment of glycaemic control and IR with NAC supplementation. Accumulating experimental evidence indicated that NAC promoted adiponectin gene expression, resulting in reduced hyperglycaemia and hyperinsulinaemia, and amelioration of IR (Ma et al. 2016, Berry et al. 2018). The principal mechanisms by which adiponectin improve insulin sensitivity seem to be through augmented fatty acid oxidation and suppression of hepatic glucose production (Lihn et al. 2005). Also, NAC could ameliorate IR through down-regulation of intracellular ROS and direct free radical scavenger actions (Keshk et al. 2020).

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 (Urakawa et al. 2003, Diniz et al. 2006). Also, recently Bilginoglu (2019) 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

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**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 analysed by a one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. *Significantly different vs. control group (p<.05). ***Significantly different vs. control group (p<.001). #Significantly different vs. MS group (p<.05). ###Significantly different vs. MS group (p<.001).
trigger ROS production (Inoguchi et al. 2000). Also, fat buildup lead to lowering of anti-oxidative enzymes which elicit ROS formation (Furukawa et al. 2004). In addition, repression of P53, which is a protector genome, reported in MS. This reduces the expression of antioxidant enzymes; glutathione peroxidase and superoxide dismutase consequently trigger cellular ROS production, leading to tissue oxidative injury (Sablina et al. 2005). Moreover, it was found that most of diseases associated with MS as IR, hypertension, obesity, dyslipidaemia might impede mitochondrial homeostasis and cause mitochondrial OS (Palmeira et al. 2018).

Figure 5. Histologic examination of representative sections of aortic tissues (×400): 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, ×400) and the NAC group (F, ×400). Increased connective tissue deposition in the TM of the aorta in the MS group (arrow) (G, ×400). Lesser connective tissue deposition in the MS + NAC group (H, ×400) than in the MS group.

Table 3. Tunica media thickness in the aorta and cardiophrin-1 expression (CT-1) in the aorta and the cardiac tissue for the studied animals’ groups.

| Variables                  | Control       | NAC           | MS            | MS + NAC       |
|----------------------------|---------------|---------------|---------------|----------------|
| Tunica media thickness (µm) | 77.09 ± 3.24  | 77.89 ± 3.16  | 219.5 ± 19.27 | 123 ± 3.13     |
| Aortic CT-1                | 1 ± 0         | 1 ± 0         | 2.63 ± 0.18   | 1.38 ± 0.18    |
| Cardiac CT-1               | 1 ± 0         | 1 ± 0         | 2.75 ± 0.16   | 1.75 ± 0.25    |

Data are presented as mean ± SE (n = 8 rats in each group), cardiophrin-1 (CT-1). Data were analysed by a one-way analysis of variance (ANOVA) followed by Tukey’s post hoc tests.

*Significantly different vs. control group (p<.05).
**Significantly different vs. control group (p<.01).
***Significantly different vs. control group (p<.001).
###Significantly different vs. MS group (p<.001).
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 (Samuni et al. 2013). It can also reinstate the pro-oxidant/antioxidant imbalance via its metal-chelation activity (Giampreti et al. 2016). 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 maintains its homeostasis (Peerapanyasut et al. 2019).

Hypertension is a main character of MS affecting about up to 85% of MS patients (Duvnjak et al. 2008). 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 (Korandji et al. 2011, Cabral et al. 2014). 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.

Figure 6. Histologic examination of representative sections of cardiac tissues (×400): 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 haemorrhage (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, ×400) and the NAC group (H, ×400). The MS group shows increased interstitial fibrosis and connective tissue deposition in the wall of the blood vessels (I, ×200). Higher power to demonstrate increased interstitial fibrosis (arrow) (J, ×400) and connective tissue deposition in the wall of the blood vessels (arrow) (K, ×400) in the MS group. No interstitial fibrosis in the MS + NAC group (L, ×400).
Also, boosting of renal expression of renin with consequent activation of angiotensin II and aldosterone formation reported in fructose fed animals (Xu et al. 2017). 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 (Komnenov et al. 2019).

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 normalisation of the arterial blood pressure (Xia et al. 2006). In addition, clinical evidence has suggested the ability of NAC to suppress the sympathetic stimulation (Jouett et al. 2016). Hence, help in blood pressure restoration in MS animals. Furthermore, NAC has been proven to restrain renin expression in the renal tissue (Thieme et al. 2016). Finally, according to Krause et al. (2018), NAC by its antioxidant activity and glutathione supply could

Figure 7. Immunohistochemical expression of cardiotrophin-1 (×400): 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).
improve hypertension in cases of increased OS associated with elevated blood pressure.

In our observations, dyslipidaemia 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. (2019) who encountered

| Table 4. Pearson’s correlation coefficient between cardiotrophin-1 expression levels in both the heart and the aorta and physiological variables and lipid profile parameters. |
| --- |
| Variables | MS | MS + NAC |
| Basal glycaemia | | |
| Cardiac CT-1 | r = 0.78 | r = 0.88 |
| p < .05 | p < .01 |
| Aortic CT-1 | r = 0.77 | r = 0.87 |
| p < .05 | p < .01 |
| Final SBP | | |
| Cardiac CT-1 | r = 0.98 | r = 0.88 |
| p < .001 | p < .01 |
| Aortic CT-1 | r = 0.76 | r = 0.83 |
| p < .05 | p < .05 |
| Final DBP | | |
| Cardiac CT-1 | r = 0.88 | r = 0.90 |
| p < .01 | p < .01 |
| Aortic CT-1 | r = 0.72 | r = 0.82 |
| p < .05 | p < .05 |
| Total cholesterol | | |
| Cardiac CT-1 | r = 0.85 | r = 0.90 |
| p < .01 | p < .01 |
| Aortic CT-1 | r = 0.89 | r = 0.86 |
| p < .01 | p < .01 |
| Triglycerides | | |
| Cardiac CT-1 | r = 0.73 | r = 0.71 |
| p < .05 | p < .05 |
| Aortic CT-1 | r = 0.82 | r = 0.67 |
| p < .05 | p = .07 |
| LDL-C | | |
| Cardiac CT-1 | r = 0.80 | r = 0.83 |
| p < .05 | p < .05 |
| Aortic CT-1 | r = 0.78 | r = 0.81 |
| p < .05 | p < .05 |
| HDL-C | | |
| Cardiac CT-1 | r = −0.77 | r = −0.78 |
| p < .05 | p < .05 |
| Aortic CT-1 | r = −0.84 | r = −0.75 |
| p < .01 | p < .01 |

p-Values with an asterisk is considered significant.

*p < .5.

**p < .01.

***p < .001.

Figure 8. Effect of NAC treatment on the Severity of fibrosis and aortic/myocardial degeneration.

Table 5. Relationship between Severity of fibrosis and aortic/myocardial degeneration and treatment status.

| Variable | MS | MS + NAC | p-Value |
| --- | --- | --- | --- |
| Severity | | | |
| Moderate/severe | 5 (62.5%) | 2 (25%) | .039 |
| Mild | 3 (37.5%) | 6 (75%) | |

n = 8 rats in each group.

*Fisher’s exact test analysis was used to compare the frequency among groups.

Table 6. Physiological and laboratory differences according to severity of fibrosis and aortic/myocardial degeneration.

| Variable | Mild | Moderate/severe | p-Value |
| --- | --- | --- | --- |
| Group (MS/MS + NAC) | | | .039 |
| (n = 9) | (n = 7) | |
| CT-1 Aorta (1/2/3) | 5/3/1 | 0/3/4 | .011 |
| Heart (1/2/3) | 2/5/2 | 1/1/5 | .049 |
| HOMA-IR (median (IQR)) | 2.8 (1.4) | 5.1 (1.3) | .038 |
| QUICKI (median (IQR)) | 0.31 (0.04) | 0.30 (0.02) | .252 |
| Final SBP (mean ± SD) | 168.00 ± 37.5 | 196.68 ± 38.5 | .201 |
| Final DBP (mean ± SD) | 137.50 ± 14.5 | 145.14 ± 11.6 | .437 |
| Basal glycaemia (mean ± SD) | 158.40 ± 19.4 | 165.65 ± 29.1 | .299 |
| T. cholesterol (mean ± SD) | 154.01 ± 18.5 | 160.94 ± 22.3 | .201 |
| TG (mean ± SD) | 129.86 ± 13.1 | 115.68 ± 12.2 | .606 |
| LDL-C (mean ± SD) | 71.44 ± 4.9 | 88.43 ± 9.1 | .142 |
| HDL-C (mean ± SD) | 25.00 ± 3.3 | 16.21 ± 3.1 | .048 |
| MDA (median (IQR)) | 0.014 (0.01) | 0.045 (0.03) | .044 |
| MDA/TAC (median (IQR)) | 0.04 (0.01) | 0.18 (0.03) | .372 |

*p-Value .05

**Mann–Whitney’s U test was used to compare the medians among groups.

***Independent t-test was used to compare the means among groups.

Table 7. Predictors of moderate/severe fibrosis and aortic/myocardial degeneration: multiple logistic regression model.

| Variable | OR (95% CI) | p-Value |
| --- | --- | --- |
| Group (MS) | 4.024 (1.584–22.797) | .043 |
| CT-1 aorta | 9.637 (1.153–86.161) | .037 |
| CT-1 heart | 3.167 (0.654–15.432) | .152 |
| HOMA-IR | 1.032 (1.002–1.546) | .048 |
| LDL | 0.888 (0.769–1.024) | .103 |
| MDA | 1.056 (1.008–2.957) | .045 |
| TAC | 0.742 (0.411–0.929) | .041 |
| MDA/TAC | 4.198 (1.354–8.252) | .43 |

OR: odd ratio; CI: confidence interval.
dyslipidaemia 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 systemically induces OS and inflammation which in turn progress forward to IR and high basal glycaemia (Park et al. 2013). Also, the IR developed by hypertriglyceridaemia 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 (Mamikutty et al. 2014). In addition, recently in a novel polygenic rat model of MS, obesity, and diabetes, Han et al. (2020) reported that gene expressions involved in lipid metabolism were dysregulated with the key proteins participating in pathogenesis of dyslipidaemia and IR were upregulated.

The co-administration of NAC revealed partial recovery of lipid profile as evidenced by normalisation of total cholesterol, triglycerides, HDL-cholesterol, and atherogenic index and decrement of LDL-cholesterol confirming the improvement of dyslipidaemia, which is supported by previous studies (Korou et al. 2014, Ma et al. 2016, Kaga et al. 2018). The lipid-lowering action of NAC in our MS animal model of hyperlipidaemia can be partially ascribed to the suppression of mRNA expression of lipogenic related enzymes (Lin et al. 2008). Also, in an experimental mouse model of high-sucrose diet feeding, NAC hampered the metabolic shifting in cardiac tissue, promoting fatty acid oxidation (Novelli et al. 2009). 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 oxidise lipoproteins and prohibit lipid intracellular uptake (Korou et al. 2014). It is possible that the decreased serum cholesterol levels in rats fed a NAC-supplementation are due to the anti-oxidative effects of NAC.

CT-1 is elevated in the myocardium and plasma of heart failure patients (López et al. 2014), and it has been proven to be related to hypertension, cardiac hypertrophy, and fibrosis both in patients (López et al. 2009, 2014, González et al. 2012) and experimental models (López-Andrés et al. 2012). Interestingly, CT-1 is up-regulated in cardiac fibroblasts and cardiomyocytes in response to metabolic, humoral, mechanical, and hypoxic stress (Hogas et al. 2017). 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. (2012) 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 (Konii et al. 2013). 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 (Tokito et al. 2013). On the other hand, decreased arterial stiffness, media thickness and vascular wall fibrosis were demonstrated in CT-1-null mice (Lopez-Andres et al. 2013).

The increase in relative heart weight in our MS animals was reported previously by Wu et al. (2018a) who showed an increase in the heart weight/body weight ratio and myocardial hypertrophy in high fructose intake mice model. Furthermore, Yan et al. (2019) 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 (DeVallance et al. 2018). Also, Martinez-Martinez et al. (2019) 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. (2017) 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 (Foltz et al. 2012, Al-Mazroua et al. 2013, Wu et al. 2018b). It has been deduced that reducing OS by NAC in pressure overload may prevent electrical remodelling and ameliorate hypertrophy in epicardial myocytes (Foltz et al. 2012). Also, down-regulation of CT-1 either in vivo or in vitro was shown to be a mechanism of cardioprotection in hypertrophied heart (Al-Mazroua et al. 2013). In addition, Wu et al. (2018b) 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 antagonised by NAC treatment through stimulation of miR-29a expression and suppression of pro-fibrotic gene TGFβ expression and secretion (Liu et al. 2019). Also, it has been demonstrated that NAC could inhibit the decrease in collagen I/III ratio which play a role in cardiac extracellular matrix (ECM) composition and cardiac hypertrophy (Ninh et al. 2019).

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. (2017) who found fibrosis, degenerative changes, neutrophil infiltration, and fat deposition in chronically fructose fed animals. Also, variable degrees of collagen fibres proliferation could be detected in routinely H&E stained heart specimens of high fructose diet-
induced MS rats (Mostafa-Hedeab et al. 2017). Furthermore, inflammatory cell infiltration and mast cell activation close to the blood vessels, and degeneration of myofibrils in cardiomyocytes with intercalated discs were reported by Acikel Elmas et al. (2019). This could be attributed to mast cell infiltration of the cardiac tissue which release chemokines, pro-inflammatory cytokines, histamine, and proteases in MS conditions (Theoharides et al. 2011). 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 inflammmasome followed by fibrosis (Kang et al. 2016). 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 glycaemia, 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 time table and a strong association of decreased CT-1 with lowering of cholesterol levels were demonstrated previously (Rendo-Urteaga et al. 2013). 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 Gkalliagkou et al. (2014), CT-1 level was positively correlated with blood pressure and the indices of arterial stiffness. In another study, positive correlation between plasma CT-1 and basal glycaemia, SBP and DBP were reported (Gamella-Pozuelo et al. 2015). 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 (Marti et al. 2018). Furthermore, Anik Ilhan et al. (2018) 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 is 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.

Although this study clearly demonstrated the development of cardio-metabolic disorders following fructose consumption in rats and the ameliorative impacts of NAC, the main limitation of this study is lacking atherosclerosis development in our model which may be due to using rat model of MS (Lozano et al. 2019).

In conclusion, NAC displayed hopeful therapeutic values in alleviation of hyperglycaemia, IR, hypertension, and OS and prevention of dyslipidaemic 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. Positive associations between CT-1 and cardiometabolic disorders make it reasonable to use CT-1 as a therapeutic target of cardio-metabolic disorders, a potential biomarker for monitoring the cardiovascular adverse consequences encountered in MS and founding early intervention and prevention strategies for MS. Also, the current study concluded that untreated MS, aortic CT-1, HOMA-IR, and OS were independent predictors for fibrosis and aortic/myocardial degeneration.

Author 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.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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Data availability statement
The datasets used and analysed during the current study available from the corresponding author on reasonable request.

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