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ORIGINAL ARTICLE

EVALUATION OF THE EFFECTS OF N-ACETYLCYSTEINE ON SERUM GLUCOSE, LIPID PROFILE, AND BODY WEIGHT IN RATS WITH FRUCTOSE-INDUCED METABOLIC SYNDROME

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Summary

Background: Overconsumption of fructose may cause metabolic syndrome (MetS). MetS pathogenesis is caused by oxidative stress, cellular malfunction, and systemic inflammation caused by hereditary and environmental factors. N-acetylcysteine (NAC) has become associated with the phrase "antioxidant." Most researchers use and test NAC with the goal of preventing or reducing oxidative stress.
Aim: To determine the positive effects of NAC on blood glucose, lipid profile, and body weight in fructose-induced metabolic syndrome in albino rats.
Materials and Methods: Forty male albino rats, 10-12 weeks old, were haphazardly divided into five groups of identical size. Group I (negative control) received tap water for 12 weeks. Group II (positive control) received a 60% w/w fructose solution (60% FS) instead of tap water for 12 weeks. Group III (NAC) received tap water and an intra-peritoneal (IP) injection of NAC (150 mg/kg/day) for 12 weeks. Group IV (protection) co-administered 60% FS orally and NAC IP injection (150 mg/kg/day) for 12 weeks. Group V (treatment) received 60% FS for 8 weeks followed by 4 weeks of drinking tap water with NAC IP injection (150 mg/kg/day). Blood samples were taken at weeks 0, 8, and 12 and were tested for serum glucose and lipid profile. All animals of each group were weighted at weeks 0, 8 and 12 of the study.
Results: Concerning serum glucose, group II showed increased glycaemia at week 8 and further elevation during week 12. Group III displayed normal glycaemia at weeks 8 and 12. In group IV, glycaemia showed elevation at week 8 followed by almost complete restoration at week 12. In group V, there was an increased glycaemia at week 8 followed by a partial restoration at week 12. Regarding lipid profile parameters, group II demonstrated a deterioration during week 8 and more worsening during week 12. There were no significant changes in group III's parameters during weeks 8 and 12. Group IV displayed a worsening in lipid profile during week 8 followed by a nearly complete improvement during week 12. During week 8, group V deteriorated, followed by a partial recovery during week 12. Concerning body weight, group II showed a weight gain at week 8 and further elevation during week 12. Group III displayed normal glycaemia at weeks 8 and 12. In group IV, glycaemia showed elevation at week 8 followed by almost complete restoration at week 12.

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In group V, there was an increased glycaemia at week 8 followed by a partial restoration at week 12. At week 8, there was a significant elevation in body weights in groups II and V compared to group I. Moreover, a significant reduction in body weight was recorded in group IV compared to group II during week 8. At week 12, a significant elevation in body weight was noticed in groups II and V compared to group I. Moreover, there was a significant reduction in body weight in group III compared to group I. On the other hand, there was a significant fall in body weight in groups IV and V compared to group II during week 12.

Conclusion: MetS was caused by a high-fructose diet, which has been shown to have a negative impact on serum glucose, lipid profiles, and body weight. Moreover, NAC has been shown to enhance these parameters in a time-dependent manner.

Key words: N-acetylcysteine; Fructose; Antioxidants; Metabolic syndrome; Serum glucose; Lipid profile; Body weight

Introduction

Fructose is a monosaccharide along with glucose and galactose, popularly known as fruit sugar. Fructose is increasingly widely utilised as a sweetener to enhance the attractiveness and appeal of meals. Dietary fructose is essentially a glucose metabolism intermediate chemical. In comparison to glucose (5.5 mmol/L), fructose (0.01 mmol/L) has a very low circulating concentration in peripheral blood (1).

High-fructose corn syrup usage has grown by at least 25% in breakfast cereals, fruit juices, bottled jams, soft beverages, and sweets during the last 30 years (2). Because it does not stimulate insulin and leptin production, which promotes satiety, increased dietary fructose contributes to caloric overconsumption through overeating and the resulting energy imbalance. Also, eating a lot of fructose is linked to MetS, which is an umbrella term for obesity, diabetes, dyslipidemia, and heart disease (3).

MetS, also known as "syndrome X," is a lipid and non-lipid metabolic illness (4) defined by a network of interconnected physiological, biochemical, clinical, and metabolic variables (5). Insulin resistance, central obesity, hypertriglyceridemia, hypertension, and dyslipidemia are among these diseases (6). Dyslipidemia is marked by high levels of plasma total cholesterol and triacylglycerol, as well as high levels of very-low-density lipoprotein cholesterol and low-density lipoprotein cholesterol and a drop in high-density lipoprotein cholesterol (7).

In research, especially metabolic research, the use of animals in experiments has been and continues to be highly essential. Animal models, often known as laboratory animals, are animals that have been handled and created to seem like or mimic a genuine object of observation. Experimental animal models are becoming increasingly useful for evaluating the efficacy of novel drugs as well as understanding their molecular background, aetiology, and mechanism of action. The outcomes of preclinical investigations, however, are not always the same as those discovered in humans (8).

The rat is a useful model to investigate how fructose affects human glucose metabolism. Rats and humans, unlike other animals, lack the intestinal enzyme that converts a significant portion of ingested fructose into glucose, so feeding rats diets high in fructose causes metabolic changes that closely mimic the human MetS (9).

An increase in oxidative stress and inflammation is also a feature of MetS. Although the pathophysiology of MetS is complicated and not completely understood, it has been postulated that a pro-oxidant/antioxidant imbalance may play a role in its progression (10). Excessive production of reactive nitrogen (RNS) and reactive oxygen (ROS) species may result in oxidative damage. to practically all biomolecules (11).

Over time, it has become clear that pharmaceutical therapies with significant antioxidant characteristics, such as NAC, are critical in reducing oxidative stress and inflammation in MetS. In the early 70s, NAC was initially

used as an antidote for paracetamol overdose treatment (12) and its enhanced capacity to replace hepatic glutathione (GSH) levels as well as lower pro-inflammatory cytokines is evident in non-alcoholic fatty liver disease (NAFLD) animal models (13).

NAC has been shown in studies to lower levels of blood sugar, as a result of its capacity to stimulate insulin manufacturing and secretion (14). By stabilising the production of atherosclerotic plaque, NAC may also assist in lessening the severity of atherosclerosis (15). According to Kaga *et al*, NAC treatment lowered cholesterol and its lipoprotein portions, as well as triacylglycerols (16).

The goal of this study was to find out how well NAC can antagonize the effects of fructose-induced MetS on serum glucose and lipid profile.

Materials and methods

Animals: A total of 40 male albino rats weighing 200–250 g were utilised in this investigation. They were taken from Mosul University's Faculty of Dentistry's animal home and housed in the same location. The animals were maintained in a room with a constant temperature of 22±2°C, 12 hour light/dark cycle, and free access to feed and water. All operations were carried out in accordance with the institutional animal research ethics committee of Mosul University's Faculty of Dentistry. This was done in perfect conformity with the 2011 standards of the National Research Council (Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press).

Experimental substances

A 60% Fructose Solution (60%FS): was made by slowly adding 600 grams of fructose to a beaker of tap water while stirring. The volume was increased to 1000 ml. Continue stirring until complete dissolution is achieved. The 60% FS was placed in plastic, foil-wrapped bottles to prevent fermentation. The fructose used in this study was manufactured in Spain by Diet Rádisson.

NAC: was delivered in ampules (300 mg N-acetyl cysteine/3 ml ampule) kept at temperatures below 25°C. The form utilized in this investigation was a Turkish medicinal product manufactured by Bilim.

Experimental design: Before fructose and NAC were administered, the rats were acclimatized for one week with a standard diet (75-80% carbohydrates, 12-20% protein, and around 4-6% of fat) and water. The standard diet is freely accessed for all groups during the whole experiment.Rats were randomly placed into five groups of eight rats each and given the following treatments for 12 weeks:

— Group I (negative control, n = 8): rats were allowed to drink tap water and given an I.P injection of distilled water (1.0 ml/kg) daily for 12 weeks; i.e., from the first week until the last week of the experiment. Distilled water is given as a placebo because it is the vehicle for NAC;
— Group II (positive control, n = 8): rats were allowed to drink 60% FS instead of tap water for the entire 12 weeks (17, 18) to induce MetS. An I.P. injection of distilled water (1.0 ml/kg/day) is also administered for the duration of the experiment;
— Group III (NAC n = 8): rats were allowed to drink tap water and received an I.P. injection of NAC (150 mg/kg/day) (19, 20) daily for the duration of the experiment.
— Group IV (protection, n = 8): rats were allowed to drink 60% FS instead of tap water and given a daily I.P. injection of NAC (150 mg/kg/day) [19, 20] from the first week until the last week of the experiment (12 weeks);
— Group V (treatment n = 8): rats were given an I.P injection of distilled water (1.0 ml/kg/day) and drank 60% FS instead of tap water for the first 8 weeks (17, 18) to induce MetS. From the 9th to the 12th week of the experiment, the group was allowed to drink tap water and given an I.P. injection of NAC (150 mg/kg/day) (19, 20).

Blood sampling: Blood samples were obtained at 11 a.m. after an overnight fast. All groups had samples taken from the retro-orbital venous plexus at weeks 0, 8, and 12 using microhematocrit capillary tubes and blood collected

in an Eppendorf tube under light ether anesthesia (21). After allowing blood samples to coagulate for 30 minutes at room temperature, they were centrifuged for 15 minutes at 3000 revolutions per minute to yield clear serum. This serum was then frozen at 20 below 0 °C so that it could be analyzed biochemically.

Biochemical analysis

Estimation of fasting serum glucose

At weeks 0, 8, and 12 of the study, blood samples were collected from dissected tails of rats and analysed with an Accu-Chek blood glucometer (Roche Diagnostics, Germany).

Estimation of the lipid profile and atherogenic index of plasma

Serum lipid profile, comprising total cholesterol (CHO), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and very-low-density lipoprotein-cholesterol (VLDL-C), were estimated at weeks 0, 8, and 12 of the study. CHO, TG, and HDL-C were evaluated using a biochemical analyzer (Smart 150 autochemistry analyzer, GenoTEK, USA). The kits used were from Giesse Diagnostics, Italy. VLDL-C was estimated by dividing plasma TG by 5, and LDL-C was obtained by deduction using the Friedewald equation (CHO=HDL-C + LDL-C + TG/5) (22). The atherogenic index of plasma (AIP) was calculated at weeks 0, 8, and 12 by the following equation: AIP = log (TG/HDL-C) (23).

Weight measuring

All animals of each group were weighted at 11 a.m. at weeks 0, 8 and 12 of the study using an electrical sensitive digital balance (SF-400C, China).

Statistical analysis

The differences between the five study groups were evaluated statistically using a one-way analysis of variance (ANOVA) and the least significant difference test (i.e., Duncan's post-hock). The significance level was set at $P<0.05$ and calculations were completed using IBM SPSS statistics software, version 22.

Results

Changes in serum glucose levels among groups in different periods of the study

At week 8, statistical analysis revealed a significant increase in serum glucose levels (159.00±6.06 mg/dl, 134.50±9.81mg/dl, and 160.43±7.65 mg/dl) in the positive control, protection, and treatment groups related to the negative control group (106.25±11.09 mg/dl). In addition, there was a significant fall in serum glucose level

Table 1. A Comparison of the effect of NAC on serum glucose level (mg/dl) in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	114.13±8.68^{a}	106.25±11.09^{a}	111.75±4.65^{ab}
Positive control	118.50±2.38^{a}	159.00±6.06^{c}	181.75±6.24^{d}
NAC	111.25±6.25^{a}	105.50±7.50^{a}	101.66±8.34^{a}
Protection	113.13±8.47^{a}	134.50±9.81^{b}	119.63±3.90^{b}
Treatment	117.13±4.80^{a}	160.43±7.65^{c}	131.65±11.23^{c}
P-value	0.59	0.00***	0.00***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P≤0.05, **P≤0.01, ***P≤0.001.

in the protection group (134.50±9.81 mg/dl) compared to the positive control group (159.00±6.06 mg/dl) during week 8. At week 12, the statistical analysis also showed a significant rise in serum glucose levels (181.75±6.24 mg/dl) in positive control and treatment groups respectively in relation to the negative control group (111.75±4.65 mg/dl). Moreover, a significant drop in serum glucose levels was noticed in the protection and treatment groups (119.63±3.90 mg/dl and 131.65±11.23 mg/dl) related to the positive control group (181.75±6.24 mg/dl) during week 12 (as shown in Table 1).

Changes in lipid profile among groups in different periods of the study

Changes in serum CHO levels

At week 8, statistical analysis demonstrated a significant rise in serum CHO levels (156.88±7.42 mg/dl and 158.38±7.74 mg/dl) in positive control and treatment groups, respectively, about the negative control group (112.13±7.69 mg/dl). Moreover, there was a significant reduction in serum CHO level (94.75±6.25 mg/dl) in the NAC group related to the negative control group (112.13±7.69 mg/dl). Additionally, there was a significant fall in serum CHO level in the protection group (114.75±9.71 mg/dl) in relation to the positive control group (156.88±7.42 mg/dl) during week 8. At week 12, statistical analysis also showed a significant rise in serum CHO levels (171.50±7.42 mg/dl and 125.63±8.46 mg/dl) in the positive control and treatment groups, respectively, in association with the negative control group (110.63±9.96 mg/dl). Moreover, there was a significant reduction in serum CHO level (90.63±5.38 mg/dl) in the NAC group compared to the negative control group (110.63±9.96 mg/dl). Also, there was a significant decrease in serum CHO levels in the protection and treatment groups (117.75±5.12 mg/dl and 125.63±8.46 mg/dl) compared to the positive control group (171.50±7.42 mg/dl) during week 12 (as shown in Table 2).

Table 2. A Comparison of the effect of NAC on serum CHO levels (mg/dl) in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	104.46±5.77^{a}	112.13±7.69^{b}	110.63±9.96^{b}
Positive control	106.75±6.60^{a}	156.88±7.42^{c}	171.50±7.42^{d}
NAC	101.25±2.75^{a}	94.75±6.25^{a}	90.63±5.38^{a}
Protection	107.25±2.50^{a}	114.75±9.71^{b}	117.75±5.12^{bc}
Treatment	107.88±9.24^{a}	158.38±7.74^{c}	125.63±8.46^{c}
P-value	0.62	0.001***	0.001***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P≤ 0.05, **P≤0.01, ***P≤0.001.

Changes in serum HDL-C level

At week 8, statistical analysis demonstrated a significant reduction in serum HDL-C levels (33.75±4.50 mg/dl and 32.25±2.63 mg/dl) in the positive control and treatment groups, respectively compared to the negative control group (44.75±3.40 mg/dl). Also, a significant elevation was shown in serum HDL-C level in the protection group (41.55±2.17 mg/dl) compared to the positive control group (33.75±4.50 mg/dl) during week 8. At week 12, Statistical analysis also showed a significant reduction in serum HDL-C level (27.41±2.11 mg/dl) in the positive control in relation to the negative control group (41.25±4.79 mg/dl). In addition, there was a significant elevation in serum HDL-C level (47.75±3.25 mg/dl) in the NAC group compared to the negative control group (41.25±4.79 mg/dl) (as shown in Table 3).

Changes in serum TG level

At week 8, statistical analysis demonstrated a significant height in serum TG levels (126.25±4.27 mg/dl and 130.50±3.42 mg/dl) in positive control and treatment groups, respectively, associated with the negative control

Table 3. A Comparison of the effect of NAC on serum HDL-C levels (mg/dl) in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	43.50±4.65[a]	44.75±3.40[b]	41.25±4.79[b]
Positive control	45.75±4.03[a]	33.75±4.50[a]	27.41±2.11[a]
NAC	42.63±3.63[a]	46.25±3.75[b]	47.75±3.25[c]
Protection	44.25±3.95[a]	41.55±2.17[b]	41.223±2.38[b]
Treatment	46.13±3.33[a]	32.25±2.63[a]	39.28±3.78[b]
P-value	0.74	0.001***	0.001***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P≤0.05, **P≤0.01, ***P≤0.001.

group (75.13±5.78 mg/dl). Also, there was a significant reduction in serum TG level (64.50±2.50 mg/dl) in the NAC group compared to the negative control group (75.13±5.78 mg/dl). Moreover, there was a significant reduction in serum TG level in the protection group (77.50±5.80 mg/dl) compared to the positive control group (126.25±4.27 mg/dl) during week 8. At week 12, the statistical analysis also showed a significant rise in serum TG levels (148.50±7.05 mg/dl and 107.75±2.99 mg/dl) in positive control and treatment groups, respectively, in comparison to the negative control group (76.25±5.56 mg/dl). Additionally, there was a significant reduction in serum TG level (60.50±4.50 mg/dl) in the NAC group compared to the negative control group (76.25±5.56 mg/dl). There was a significant fall in serum TG levels in the protection and treatment groups (81.00±3.47 mg/dl and 107.75±2.99 mg/dl) compared to the positive control group (148.50±7.05 mg/dl) during week 12 (as shown in Table 4).

Table 4. A Comparison of the effect of NAC on serum TG levels (mg/dl) in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	72.63±3.86[a]	75.13±5.78[b]	76.25±5.56[b]
Positive control	75.13±2.78[a]	126.25±4.27[c]	148.50±7.05[d]
NAC	74.25±3.75[a]	64.50±2.50[a]	60.50±4.50[a]
Protection	71.00±6.06[a]	77.50±5.80[b]	81.00±3.47[b]
Treatment	74.25±3.86[a]	130.50±3.42[c]	107.75±2.99[c]
P-value	0.67	0.001***	0.001***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P≤0.05, **P≤ 0.01, ***Pp≤0.001.

Changes in serum LDL-C level

At week 8, statistical analysis demonstrated a significant increase in serum LDL-C levels (97.88±11.17 mg/dl and 100.03±9.59 mg/dl) in the positive control and treatment groups, respectively, compared to the negative control group (52.35±9.89 mg/dl). Also, there was a significant reduction in serum LDL-C level (35.60±5.00 mg/dl) in the NAC group in relation to the negative control group (52.35±9.89 mg/dl). A significant reduction in serum LDL-C level was recorded in the protection group (57.70±8.73 mg/dl) compared to the positive control group (97.88±11.17 mg/dl) during week 8. At week 12, the statistical analysis also showed a significant elevation in serum LDL-C level (114.39±7.9 mg/dl) in the positive control in relation to the negative control group (54.13±11.48 mg/dl). In addition, there was a significant reduction in serum LDL-C levels (30.78±4.23 mg/dl) in the NAC group in association with the negative control group (54.13±11.48 mg/dl). There was a significant fall in serum LDL-C levels in the protection and treatment groups (60.33±5.25 mg/dl and 64.80±9.23 mg/dl) related to the positive control group (114.39±7.9 mg/dl) during week 12 (as shown in Table 5).

Table 5. A Comparison of the effect of NAC on serum LDL-C levels (mg/dl) in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	44.80±9.70^{a}	52.35±9.89^{b}	54.13±11.48^{b}
Positive control	46.54±6.69^{a}	97.88±11.17^{c}	114.39±7.9^{c}
NAC	43.78±4.63^{a}	35.60±5.00^{a}	30.78±4.23^{a}
Protection	48.80±3.56^{a}	57.70±8.73^{b}	60.33±5.25^{b}
Treatment	46.90±9.40^{a}	100.03±9.59^{c}	64.80±9.23^{b}
P-value	0.90	0.001***	0.001***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P≤0.05, **P≤ 0.01, ***P≤0.001.

Changes in serum VLDL-C level

At week 8, statistical analysis demonstrated a significant rise in serum VLDL-C levels (25.25±0.85 mg/dl and 26.10±0.68 mg/dl) in the positive control and treatment groups, respectively, in relation to the negative control group (15.03±1.16 mg/dl). Moreover, there was a significant reduction in serum VLDL-C level (12.90±0.71 mg/dl) in the NAC group compared to the negative control group (52.35±9.89 mg/dl). A significant reduction in serum VLDL-C level was shown in the protection group (15.50±1.16 mg/dl) related to the positive control group (25.25±0.85 mg/dl) during week 8. At week 12, the statistical analysis also showed a significant elevation in serum VLDL-C level (29.70±1.41 mg/dl and 21.55±0.6 mg/dl) in the positive control and treatment groups, respectively, in relation to the negative control group (15.25±1.11 mg/dl). There was a significant reduction in serum VLDL-C levels (12.10±1.27 mg/dl) in the NAC group compared to the negative control group (15.25±1.11 mg/dl). Also, there was a significant fall in serum VLDL-C levels in the protection and treatment groups (16.20±0.69 mg/dl and 21.55±0.6 mg/dl) compared to the positive control group (29.70±1.41 mg/dl) during week 12 (as shown in Table 6).

Table 6. A Comparison of the effect of NAC on serum VLDL-C levels (mg/dl) in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	14.53±0.77^{a}	15.03±1.16^{b}	15.25±1.11^{b}
Positive control	15.03±0.60^{a}	25.25±0.85^{c}	29.70±1.41^{d}
NAC	14.85±1.06^{a}	12.90±0.71^{a}	12.10±1.27^{a}
Protection	14.20±1.21^{a}	15.50±1.16^{b}	16.20±0.69^{b}
Treatment	14.85±0.77^{a}	26.10±0.68^{c}	21.55±0.6^{c}
P-value	0.71	0.001***	0.001***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P≤0.05, **P≤0.01, ***P≤0.001.

Changes in AIP values

At week 8, statistical analysis demonstrated a significant elevation in AIP values (0.58±0.06 and 0.61±0.04) in positive control and treatment groups respectively in comparison to the negative control group (0.23±0.07). Moreover, there was a significant fall in serum AIP value in protection group (0.27±0.06) compared to the positive control group (0.58±0.06) during week 8. At week 12, statistical analysis also showed a significant elevation in AIP value (0.73±0.030.44±0.05) and in the positive control and treatment groups, respectively, compared to the negative control group (0.27±0.05). Moreover, there was a significant reduction in AIP value (0.10±0.02) in NAC group

in relation to the negative control group (0.27±0.05). It is important to notice that there was a significant reduction in AIP values (0.29±0.04 and 0.44±0.05) in protection and treatment groups in association to positive control group (0.73±0.03) (as shown in Table 7).

Table 7. A Comparison of the effect of NAC on AIP values in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	0.22±0.03^{a}	0.23±0.07^{ab}	0.27±0.05^{b}
Positive control	0.22±0.04^{a}	0.58±0.06^{c}	0.73±0.03^{d}
NAC	0.24±0.02^{a}	0.15±0.02^{a}	0.10±0.02^{a}
Protection	0.20±0.06^{a}	0.27±0.06^{b}	0.29±0.04^{b}
Treatment	0.21±0.04^{a}	0.61±0.04^{c}	0.44±0.05^{c}
P-value	0.78	0.001***	0.001***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P≤0.05, **P≤0.01, ***P≤0.001.

Changes in body weight among groups in different periods of the study.

At week 8, statistical analysis demonstrated a significant elevation in body weights (323.33±8.08 g and 321.88±7.60 g) in positive control and treatment groups, respectively compared to the negative control group (303.13±5.89 g). Moreover, a significant reduction in body weight was recorded in protection group (307.63±12.99 g) compared to the positive control group (323.33±8.08 g) during week 8. At week 12, statistical analysis also showed a significant elevation in body weights (374.13±7.40 g and 349.18±12.70 g) in the positive control and treatment groups, respectively compared to the negative control group (332.75±5.74 g). Moreover, there was a significant reduction in body weight (316.65±6.45 g) in NAC group compared to the negative control group (332.75±5.74 g). On the other hand, there was a significant fall in body weight in protection and treatment groups (336.88±7.06 g and 349.18±12.70 g) compared to the positive control group (374.13±7.40 g) during week 12 (as shown in Table 8).

Table 8. A Comparison of the effect of NAC on body weight (g) in fructose-induced MetS in rats among five groups at three follow-up periods.

Group	Week 0	Week 8	Week 12
Negative control	220.75±6.34^{a}	303.13±5.89^{ab}	332.75±5.74^{b}
Positive control	216.50±6.35^{a}	323.33±8.08^{c}	374.13±7.40^{d}
NAC	228.75±8.75^{a}	292.50±7.50^{a}	316.65±6.45^{a}
Protection	222.75±9.67^{a}	307.63±12.99^{b}	336.88±7.06^{bc}
Treatment	225.88±13.16^{a}	321.88±7.60^{c}	349.18±12.70^{c}
P-value	0.47	0.00***	0.00***

The data is presented as the mean±standard deviation (SD). Different small letters represent the differences in significance among groups. *P ≤0.05, **P≤0.01, ***P≤0.001.

Discussion

The current study looked at the effects of fructose overconsumption on serum glucose and lipid profile, as well as the protective effect of NAC as an antioxidant that counteracts fructose's negative effects on these parameters. In laboratory animals, there is an undeniable link between chronic high dietary fructose intake and increased body weight, energy intake, adiposity, hypertension, hypertriglyceridemia, hyperlipidemia , glucose intolerance, and decreased insulin sensitivity, all of which lead to MetS (1).

Fructose can affect pancreatic β-cell function and mass in rat islets, according to Li, Jian-Mei *et al* (24), by impairing leptin signaling and activating protein kinase B (PKB)/Forkhead box protein (Fox) O1. A condition of hepatic insulin resistance resulting from a prolonged exposure to fructose may be linked to the process underlying the alteration of glucose metabolism. Fructose promotes de novo lipogenesis in the liver, causes endoplasmic reticulum stress and inflammation, and reduces hepatocyte insulin sensitivity (25, 26). All these narratives can interpret our findings of hyperglycemia in the positive control, treatment, and protection groups.

The capacity of NAC's thiol group to interact with ROS and RNS is what gives it its direct antioxidant effect (27). However, it is believed that NAC has a direct antioxidant action contrary to specific oxidative species, such as •NO2 and hypohalous acids (HOX), depending on its relative quantity in relation to other thiols (28).

Because of its capacity to raise intracellular cysteine levels and therefore GSH levels, NAC is crucial as a powerful antioxidant. In situations of xenobiotic intoxication, such as paracetamol, or in diseases associated to GSH shortage, NAC is one of the primary techniques to limit the damage produced by oxidative stress via the preservation of their levels in different tissues. Since the separate use of GSH and cysteine failed to increase intracellular GSH levels, they were combined (29). NAC also offers an indirect antioxidative impact through another mechanism including its potential for reducing. NAC is capable of replenishing systemic pools of low-molecular-weight (LMW) thiols and reduced protein sulfhydryl groups, which are implicated in the regulation of the redox state (28).

Additionally, NAC has anti-inflammatory properties by blocking the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B), which is implicated in the immunological response and inflammatory cascade to OS. NAC inhibits the nuclear activation and translocation of the transcription factor NF-B, which regulates pro-inflammatory gene expression (27). NAC inhibits the release of inflammatory cytokines IL-1, IL-6, and TNF from lipopolysaccharide-activated macrophages (30).

During week 12, it was evident that the glucose levels in the protection and treatment groups were lower than those in the positive control group by a significant margin. This phenomenon is explained by Lenzen, who reported that NAC's anti-hyperglycemic effect may be mediated by enhancing the antioxidant defence. Pancreatic β-cells are highly susceptible to oxidative stress and its associated damage due to their low antioxidant capacity (31).

In adipose tissue and muscle, fructose is phosphorylated by the enzyme hexokinase to form fructose 6-phosphate, which then participates in glycolysis. In contrast, when glucose is metabolized in the liver, glucokinase interacts instead of hexokinase, with the disadvantage that it can only phosphorylate glucose. For this reason, fructose enters the fructose-1-phosphate pathway (32).

Fructokinase converts fructose to fructose-1-phosphate by utilising ATP to metabolise fructose (33). Wong *et al*. (1) reported that fructose-1-phosphate is split into dihydroxyacetone phosphate and glyceraldehyde without the transformation of glucose to fructose-1,6-bisphosphate, the first regulated step of glycolysis (1).

According to Carvalho *et al*. (34), phosphofructokinase is the negative regulator of glucose metabolism. So fructose will continuously enter the pathway of glycolysis. The glycolysis process then converts fructose-1,6-bisphosphate to pyruvate. Pyruvate enters the cellular respiration chain or produces acetyl-CoA, which provides carbon skeletons for acyl-coenzyme A synthesis (acyl-CoA). Dihydroxyacetone phosphate is transformed into glycerol 3-phosphate, which binds to acyl-CoA molecules and then changes to acyl glycerol, which is used to synthesise TG or, with the addition of Apolipoprotein B (Apo-B) molecule, to produce VLDL (34). These results back up what we saw in the treatment and positive control groups, where VLDL-C and TG levels went up a lot.

In TG-rich conditions, VLDL produces small dense low-density lipoprotein (sdLDL). The lipoprotein lipase (LPL) enzyme first converts VLDL particles to LDL classes III and IV. Cholesteryl ester transfers protein (CETP) enzyme then transfers TG to sdLDL particles with the help of hepatic LPL, increasing LDL particle levels. When the level of TG is low, VLDL particles change into intermediate-density lipoprotein (IDL) and large LDL (35).

These results are in line with the fact that LDL-C levels rose significantly in both the positive control and treatment groups. This is because eating too much fructose increases the production of VLDL, which is then turned into LDL (as we've already talked about).

Hepatic LPL is a key enzyme in the clearance of TG-containing lipoproteins from the circulation, and insulin resistance in patients with MetS is linked to decreased LPL activity, which lowers HDL levels (36). This comes in line with our finding of a significant fall in HDL-C in the positive control and treatment groups.

All the above-mentioned impacts of fructose overconsumption on LDL-C, VLDL-C, TG, and HDL-C can give us an interpretation of the significant rises in CHO and AIP.

NAC has been shown in studies to increase insulin secretion. The existence of insulin raises the activity of LPL, which catalyses the split of triacylglycerol ester bonds, increasing VLDL-C clearance (16). This can interpret our finding of declined TG, VLDL-C and consequently LDL-C in treatment and protection groups.

Furthermore, Hang *et al*. (37) found that NAC was effective in depressing triglyceride accumulation both *in vitro* and *in vivo*, and this is linked to its ability to maintain mitochondrial function. This is consistent with our finding of lower TG levels in treatment and protection.

HDL-C levels increased in treatment and protection groups. Almeida *et al.* back up these findings (38). Also, high insulin levels increase the activity of lecithin cholesterol acyltransferase (LCAT) and the enzyme responsible for extracellular cholesterol esterification. This improves the efficiency of reverse cholesterol transport and shows an inverse relationship with heart attacks (39).

So, the NAC was able to lower AIP in both the protection and treatment groups because it was possible to control the lipid profile. Yang *et al*. also found that an improved AIP is linked to low antioxidant activity, which backs up this finding. (40). This also comes in line with the lower CHO levels in these two groups.

According to Zalewska *et al*. (41), the earlier the start of NAC supplementation, the more satisfactory the results. Mazzoli *et al*. (42) also found that removing fructose from the diet reverses mitochondrial dysfunction, oxidative stress and inflammation in the hippocampus. This can help us interpret our finding at the end of the study that the protection group's serum glucose level and lipid profile are nearly completely restored compared to that of the treatment group, which shows partial restoration.

Numerous studies demonstrate how consuming soft drinks causes weight gain (43, 44, 45). The processes clarifying this include the control of insulin, leptin, and ghrelin and are independent of the caloric diet surplus (46). Consuming fructose results in a minimum insulin release that is inadequate to induce leptin synthesis in adipocytes. A lack of leptin makes people eat more, which delays the activation of signals that tell the body it's full (47).

Additionally, fructose consumption might be a factor in the absence of ghrelin secretion inhibition. This is always brought on by the decreased insulin secretion and increased glycaemic response that follow fructose consumption (46). Finally, the liver experiences an ATP deficit as a result of the uncontrolled phosphorylation of fructose. In turn, when ATP is used up, it makes you want to eat more, which makes you gain weight (48).

All these theories can explain our results of significant weight gain in the positive control and treatment groups at weeks 8 and 12 of the current study compared to the negative control group for the same week.

This also comes in line with the findings of Carvalho *et al*. (34) who claimed that insulin and leptin are responsible for endocrine signaling in the central nervous system (CNS) in the long-term regulation of energy balance. If you eat a lot of high fructose for a long time, your daily caloric intake may go up while your energy use goes down. This could lead to weight gain and obesity, which are caused by less insulin and leptin signaling in the CNS.

In an *in vitro* experiment, NAC treatment of cultured adipocytes inhibited the production of ROS and lipid accumulation (49). Additionally, NAC supplementation showed a weight reduction effect in experimental models

of diet-induced obesity (50, 51). According to Ma *et al*. (50), NAC administration prevents lipid accumulation in brown adipose tissue, which is important for mobilizing lipid utilization and thermogenesis.

Additionally, they found that NAC therapy increased the expression of thermogenic genes in treated rats, indicating that it may increase energy expenditure. Moreover, Shen *et al*. (51) claimed that a vicious cycle of reduced OS and increased motor activity, which helps to reduce body fat and weight, was responsible for the reduction of body weight with NAC treatment.

All these notes come in line with our finding of a significant decrease in weight in the protection group at week 8 and in the protection and treatment groups at week 12 in relation to the positive control group.

Although the efficacy of NAC in counteracting deleterious fructose effects is evidenced by the outcomes of co-administration of these two materials in group IV (protection) if compared to the fructose group (positive control), it is important to know that results obtained in the treatment group must be taken with precaution as these results are attributed not only to NAC administration but also to fructose withdrawal.

Conclusion

High fructose diet consumption induced MetS in rats and had negative effects on serum glucose, lipid profile, and body weight. Furthermore, this study sheds light on the potential use of NAC to alleviate the main symptoms of MetS and partially or completely restore these parameters. More importantly, the magnitude of the NAC effect was time-dependent.

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Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the Research Ethical Committee and Scientific Committee in the Department of Dental Basic Science of the College of Dentistry/ University of Mosul with approval number (UoM.Dent/A.L.19/22).

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