Histological and Molecular Study of N-acetyl Cysteine's Effects on Salivary Glands in Fructose-Induced Metabolic Syndrome in Albino Rats

Original Article

Auss Z. Yahya[1], Ghada A. Taqa[2] and Muhammad A. Alkataan[3]

[1]*Ministry of Health, Ninevah Health Directorate, Mosul, Iraq*

[2]*Department of Dental Basic Sciences, College of Dentistry. University of Mosul, Mosul, Iraq*

[3]*Department of Biochemistry, College of Medicine, University of Ninevah, Mosul , Iraq*

ABSTRACT

Introduction: A diet high in fructose can result in metabolic syndrome (MetS). MetS is caused by a combination of hereditary and acquired variables that induce oxidative stress, cellular malfunction, and systemic inflammation. N-acetylcysteine (NAC) is the gold standard for treating paracetamol toxicity . Additionally, NAC became the ideal of a "antioxidant" throughout time. The majority of researchers use and evaluate NAC with the hope of preventing or alleviating oxidative stress.

Aim of the Work: To evaluate the possible ameliorating effects of NAC on the salivary glands of fructose-induced MetS in rats.

Materials and Methods: Forty male albino rats, 10–12 weeks old, were randomly divided into five equal groups. Group I (negative control) received tap water for 12 weeks. Group II (positive control) received 60%w/w fructose syrup instead of tap water for 12 weeks. Group III (NAC) received tap water and (IP) injection of NAC (150 mg/kg/day) for 12 weeks. Group IV (protection) took 60%FS by mouth along with an NAC 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). Rats were euthanized at the end of the 12 weeks. Salivary glands were dissected and examined histologically and for mitochondrial DNA copy number (mtDNA-CN).

Results: Histologically, group II exhibited eosinophilia, atrophy and necrosis. Group III displayed a normal architectural picture. In group IV, there was a normal architectural picture with mild epithelial degeneration. In group V, most tubules returned to their glandular appearance, but there was still eosinophilia and some degeneration and necrosis. Molecularly, in group II, there was a significant decline in mtDNA-CN. Group III showed the highest mtDNA-CN among all groups. In group IV, a significant increase in mtDNA-CN was observed, but less than in group III. Group V also showed a significant increase, but less than group IV.

Conclusion: High fructose diets induce MetS and are proven to harm the salivary glands. NAC, on the other hand, has been shown to alleviate MetS and protect and cure salivary glands from MetS's deleterious effects in a time-dependent manner.

Received: 14 April 2022, **Accepted:** 28 April 2022

Key Words: Antioxidants, fructose, metabolic syndrome, N-acetyl cysteine, salivary glands.

Corresponding Author: Ghada A. Taqa, PhD, DDepartment of Dental Basic Sciences, College of Dentistry. University of Mosul, Mosul, Iraq, **Tel.**: 07702091219, **E-mail:** ghadataqa@uomosul.edu.iq

ISSN: 1110-0559, Vol. 46, No. 3

INTRODUCTION

Fructose is a naturally occurring sugar found in fruits and honey. Due to its sweetness, palatability, and ability to enhance the taste of processed foods and beverages, it is widely used today. Consumption of high-fructose corn syrup has increased by at least 25% over the last three decades in fruit juices, canned jams, soft drinks, breakfast cereals, and candies[1].

Increased dietary fructose contributes to caloric overconsumption associated with overeating and the resulting energy imbalance, as it does not stimulate insulin and leptin secretion, which promotes satiety. Additionally, fructose consumption may be a risk factor for obesity, diabetes, dyslipidemia, and cardiovascular morbidity, all of which are referred to collectively as MetS[2].

MetS is a collection of clinical findings that includes visceral adiposity, insulin resistance, elevated triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C), and hypertension. These findings are associated with an increased risk of developing type 2 diabetes mellitus (T2DM) and atherosclerotic cardiovascular disease (ASCVD)[3].

MetS is associated with an increase in oxidative stress and inflammation. While the pathogenesis of MetS is extremely complex and not fully understood, it has been suggested that a prooxidant/antioxidant imbalance may play a significant role in its development[4]. Excessive production of reactive oxygen and nitrogen species (ROS and RNS) can cause oxidative damage to virtually all biomolecules[5].

 DOI: 10.21608/ejh.2022.133355.1673

A mitochondrion is a self-replicating, semi-autonomous organelle found in the cytoplasm of eukaryotic cells.[6] In comparison to nuclear genomic DNA, mtDNA has a high mutation rate due to constant exposure to mutagenic oxygen radicals and the absence of DNA repair mechanisms. These characteristics of mtDNA suggest that it may play a role in the pathophysiology of a variety of diseases.[7] The role of mtDNA-CN in MetS has only been studied in a few studies. Reduced mtDNA-CN has been linked to MetS components in the general population,[8] and patients with MetS have lower mtDNA-CN than controls, according to some studies.[9] In addition, Fazzini *et al*.[10] concluded in 2021 that mtDNA-CN has an inverse relationship with MetS risk.

MetS also has an effect on the salivary glands' secretion activity, altering both the quantitative and qualitative composition of unstimulated and stimulated saliva.[11]

Numerous researchers have concentrated on screening a variety of pharmacological compounds for their ability to ameliorate MetS.[12,13,14,15] It has become clear over time that pharmacological interventions with potent antioxidant properties, such as NAC, are critical for reducing oxidative stress and inflammation in MetS. NAC was developed in the early 1970s as a treatment for paracetamol overdose,[16] and its enhanced capacity to replenish hepatocellular glutathione (GSH) levels while also reducing pro-inflammatory cytokines is evident in experimental models of non-alcoholic fatty liver disease (NAFLD).[17]

The purpose of this study was to determine whether NAC can mitigate the detrimental effects of fructose-induced MetS on the salivary glands.

MATERIALS AND METHODS

Animals

This study used forty male albino rats that were 10–12 weeks old and weighed 200–250 g. They were obtained from the faculty of Dentistry's animal house at Mosul University, Iraq, and are currently housed there. The animals were kept at a room temperature of 22±2°C with 12-hour light/dark cycles and were given free access to food and water. All procedures followed the guidelines of the Faculty of Dentistry's institutional animal research ethics committee. This was done strictly in accordance with the National Research Council's 2011 requirements (Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press).

Experimental substances

60% FS was prepared by placing 600 g fructose in a beaker and gradually adding tap water while stirring. The volume was increased to 1000 ml. Stirring was continued until the solution was completely dissolved. To avoid fermentation, the 60%FS was placed in plastic, foil-enveloped bottles. The fructose used in this study was manufactured in Spain by Diet Rádisson.

NAC was available in the form of ampules (300 mg of N-acetyl cysteine/3ml ampule) that should be stored at a temperature no higher than 25°C. The formulation used in this study was a Bilim Pharmaceuticals product from Turkey.

Experimental design

Before fructose and NAC intervention, the rats were acclimatized for one week and fed a standard diet and water. Rats were randomly assigned to five groups of eight rats each and given the following treatment for 12 weeks:

- **Group I (negative control n=8):** Rats were allowed to drink tap water and received an intraperitoneal injection of distilled water (1.0 ml/kg) daily for 12 weeks, from the first to the last week of the experiment;
- **Group II (positive control n = 8):** Rats were given 60% FS instead of tap water for the entire 12 weeks (but not less than 8 weeks)[18,19] to induce MetS. Additionally, an I.P. injection of distilled water (1.0ml/kg/day) is given throughout the duration;
- **Group III (NAC n=8):** Rats were allowed to drink tap water and were daily given an I.P. injection of NAC (150mg/kg/day)[20,21].
- **Group IV (protection n=8):** Rats were given 60%FS instead of tap water and daily I.P. injections of NAC (150mg/kg/day)[20,21] from the first to the final week of the experiment (12 weeks);
- **Group V (treatment n=8):** Rats received an IP injection of distilled water (1.0ml/kg/day) and were given 60% FS instead of tap water for the first eight weeks[18,19]. From the ninth to the twelfth weeks of the experiment, the group was given tap water and an I.P. injection of NAC (150mg/kg/day).[20,21]

Specimen collection

Two hours following the last treatment , all animals of each group were placed on under light ether anesthesia and sacrificed and salivary glands were excised. Two specimens are derived from each rat. The first specimen is placed in 10% buffered formalin for histological examination. The second specimen is placed in Phosphate buffer saline (FBS) (0.01M, pH=7.4) for gene expression testing (measuring mtDNA-CN).

Evaluation methods

Histological examination

Specimens from salivary glands were processed for light microscopical examination as follows:[22]

1. Fixation of the specimens in 10% buffered formalin then rinsed with water and dehydrated through ascending alcohol concentration (70%/24hrs, 80%/1hr, 90%/1hr and 100% for two exchanges 1hr /each step).

2. The specimens were cleared by two exchanges of xylene, 10 minutes / each change.
3. Infiltration of the specimens with clear white paraffin wax in an oven At 58°C, two exchanges 2hrs/ each step then embedded in paraffin wax that was blocked in the tissue cassette.
4. The paraffin block was sliced by a rotary microtome at 4-6µm sections, then put in the circular water bath at 45C° and finally by using an adhesive agent (glycerol with egg albumin 1volume/1volume) the sections were attached to a glass slide.
5. De-parafinization: the slide is placed into xylene, and then heated in oven at 56C° for 45-60 minutes and then it placed in the second change of xylene for 5 minutes.
6. Rehydration: the section was gradually rehydrated by absolute alcohols as follows :(100%) two changes of 2 minutes each then 90%, 70% of alcohol 2 minutes of each, respectively.
7. Placed in distilled water for 5 minutes.
8. Staining by Hematoxylin and Eosin:

o Sections were placed in a glass staining jar that was full of Hematoxylin for 5 minutes. Then just once dipped in tap water, and then placed into a jar of Eosin for 1 minute.

- Dehydration by ascending concentration of alcohol 95% for 2 minutes, then 100% alcohol, 2 exchanges, 2 minutes each.
- Clearing: done by xylene 2 exchanges, 2 minutes each.
- After the slide was stained and dried, the section was covered with a coverslip by using D.P.X. as a mounting agent.
- Light microscope examination was done by two histopathological specialists.

DNA extraction and quantitative polymerase chain reaction

Salivary gland samples were lysed with 10% Proteinase K in PBS, 200 µl of buffer-AL, vortexed thoroughly, and incubated at 56 oC in a water bath for 10 minutes. Following incubation, 200 µl of ethanol of molecular grade was added. This is how it worked: After a thorough mix, the solution was transferred to an empty spin column and centrifuged at 5000 RPM for 1 minute.

The flow through was discarded following centrifugation, leaving total DNA bound to the column membrane. After washing the column membrane with 500 µl of wash1 washing buffer, the column was centrifuged at 3500 rpm for 1 minute. The flow through was discarded once more and 500 µl of wash2 washing buffer was added. The column was centrifuged once more for 3 minutes at 5000 RPM. The flow through has been omitted.

After that, the column was placed in a 1.5 mL tube. Water free of DNase and RNase (50 µl) was added carefully to the center of the spin column membrane and allowed to soak for 2 minutes. After centrifuging the column for 1 minute at 10,000 rpm, the eluted DNA was transferred to ice. Total DNA was isolated using the Addbio DNA extraction kit (Korea). The concentration and purity of the extraction product were determined using an IMPLEN nanophotometer® N60/N50. Primers were designed using NCBI blast software and synthesized as forward and reverse sequences. DNA amplification experiment was carried out using qPCR master mix (Promega Corporation, USA, GoTaq® qPCR) using the PCRmax device. Data was analyzed using Eco-Study software.

Statistical analysis

SPSS version 21 for Windows software was used to conduct the statistical analysis. Mean and standard deviation are descriptive statistics for data (SD). The t-test was used to analyze the data from five groups.

RESULTS

Histological results

Microscopical examination of tissue sections revealed the presence of pathological changes that varied significantly between groups, as illustrated below:

- **Group I (negative control):** The negative control group's rat submandibular salivary gland exhibits normal architecture, with granular, convoluted tubules, serous and mucous acini, and striated ducts. (Figures 1,2).
- **Group II (positive control):** The positive control group's rat submandibular salivary gland exhibits an increase in eosinophilia of granular convoluted tubules, atrophy and necrosis of the epithelium lining them, congestion of blood vessels, and the absence of others. (Figures 3,4).
- **Group III (NAC):** The normal architecture of the rat submandibular salivary gland is represented by granular, convoluted tubules, serous and mucous acini, striated ducts, and interlobular ducts. (Figures 5,6).
- **Group IV (protection):** The rat submandibular salivary gland of the protection group exhibits normal architecture of serous and mucous acini and striated ducts, but mild epithelial cell degeneration lining granular convoluted tubules. (Figures 7,8).
- **Group V (treatment):** The rat submandibular salivary gland of the treatment group exhibits normal architecture of the majority of granular convoluted tubules that have been restored to granular appearance with residual eosinophilia, degeneration, and necrosis in a few of them, as well as normal serous acini and striated ducts. (Figures 9, 10).

Changes in mtDNA expression of salivary glands

Significant changes in mtDNA-CN were noticed among different groups of the study, as demonstrated below: (Figure 11)

There was a significant decline in mtDNA-CN in group II (positive control) compared to groups I, III, IV and V (negative control, NAC, protection and treatment respectively).Concerning group III (NAC), a significant increase in mtDNA-CN was noticed compared to groups I, II, IV and V (negative control, positive control, protection and treatment respectively).

Regarding group IV (protection), there was a significant decrease in mtDNA-CN compared to NAC group. However, there was a significant increase in relation to negative control, positive control and treatment groups. Moreover, a significant decline in mtDNA-CN was observed in group V (treatment) compared to groups III and IV (NAC and protection respectively). However, a significant elevation was noticed compared to groups I and II, (positive control and negative control respectively).

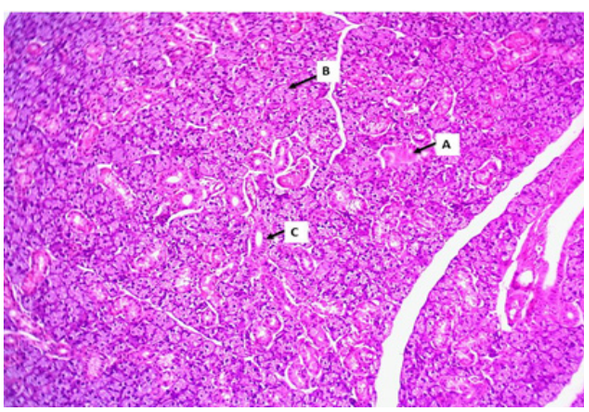

Fig. 1: A photomicrograph of a section of the submandibular salivary gland of a negative control group (Group I) shows normal architecture represented by granular convoluted tubules (A), serous and mucous acini (B) and striated ducts (C). (H & E stain x 100).

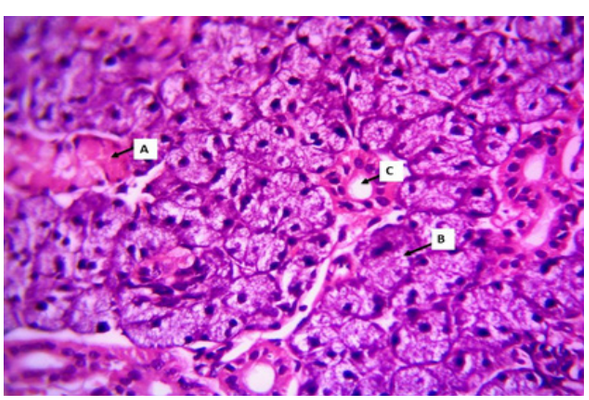

Fig. 2: A photomicrograph of a section of the submandibular salivary gland of a negative control group (Group I) shows normal architecture represented by granular convoluted tubules (A), serous and mucous acini (B) and striated ducts (C). (H & E stain x 400).

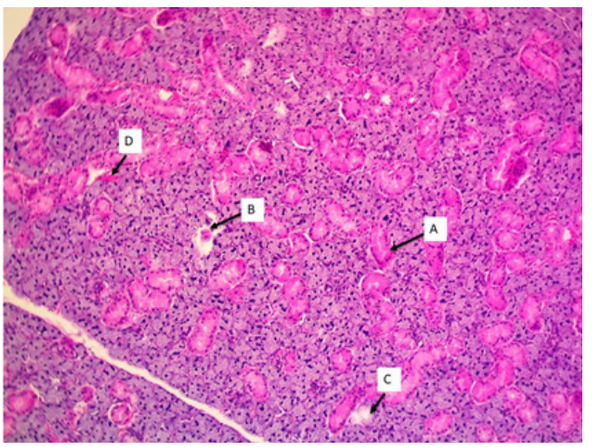

Fig. 3: A photomicrograph of a section of the submandibular salivary gland of a positive control group (Group II) shows increased eosinophilia of granular convoluted tubules (A), with atrophy (B) and necrosis (C) of the epithelium lining them and congestion of blood vessels (D). (H & E stain x 100).

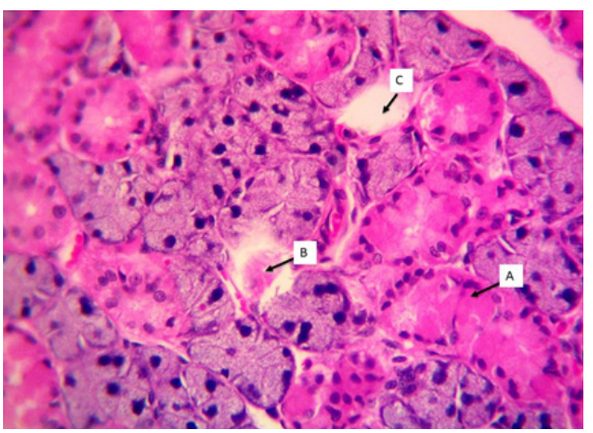

Fig. 4: A photomicrograph of a section of the positive control group's submandibular salivary gland (Group II) shows increased eosinophilia of granular convoluted tubules (A), atrophy and necrosis of the epithelium lining them (B), and a complete absence of others (C).(H & E stain x 400).

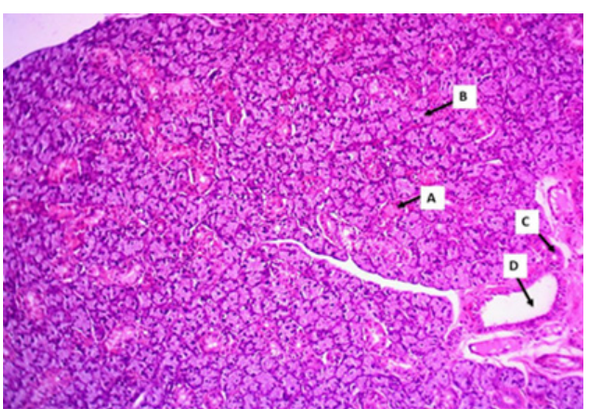

Fig.5: A photomicrograph of a section of the submandibular salivary gland of the NAC group (Group III) shows normal architecture represented by granular convoluted tubules (A), serous and mucous acini (B), striated ducts (C), and interlobular ducts (D). (H&Estainx100).

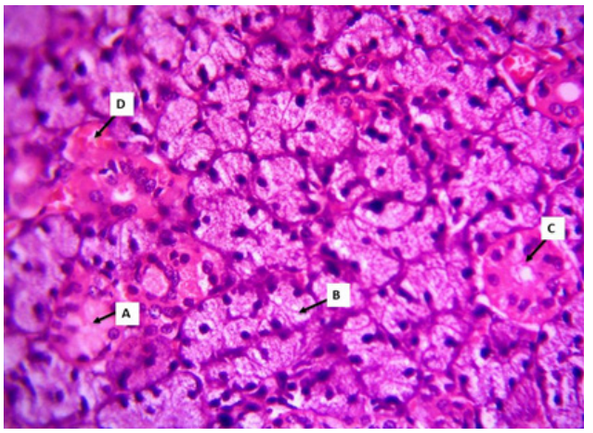

Fig. 6: A photomicrograph of a section of the submandibular salivary gland of the NAC group (Group III) shows normal architecture represented by granular convoluted tubules (A), serous and mucous acini (B), striated ducts (C), and interlobular ducts (D). (H & E stain x 400).

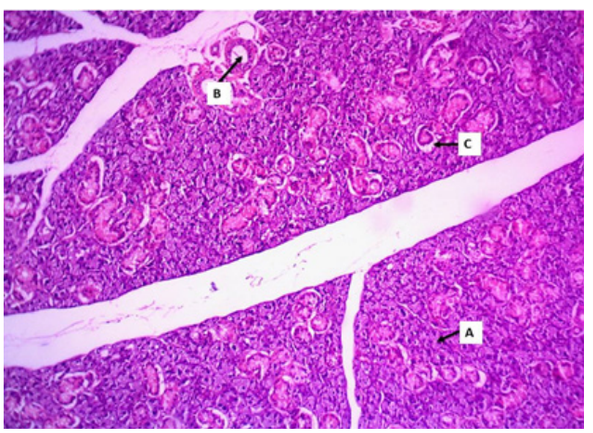

Fig. 7: A photomicrograph of a section of the submandibular salivary gland of protection group (Group IV) shows the normal architecture of serous and mucous acini (A) and striated ducts (B) with mild degeneration of epithelial cells lining granular convoluted tubules (C). (H & E stain x 100).

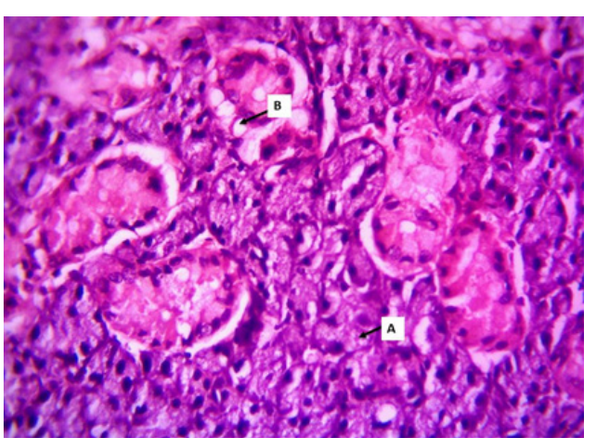

Fig. 8: A photomicrograph of a section of the submandibular salivary gland of protection group (Group IV) shows the normal architecture of serous and mucous acini (A) with mild degeneration of epithelial cells lining granular convoluted tubules (B). (H & E stain x 400).

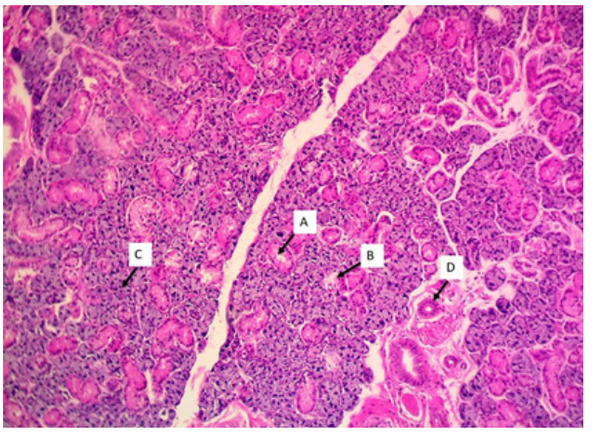

Fig. 9: A photomicrograph of a section of the submandibular salivary gland of a treatment group (Group V) shows normal architecture of most granular convoluted tubules (A) with degeneration and necrosis of a few of them (B), normal serous acini (C) and striated ducts (D). (H & E stain x 100).

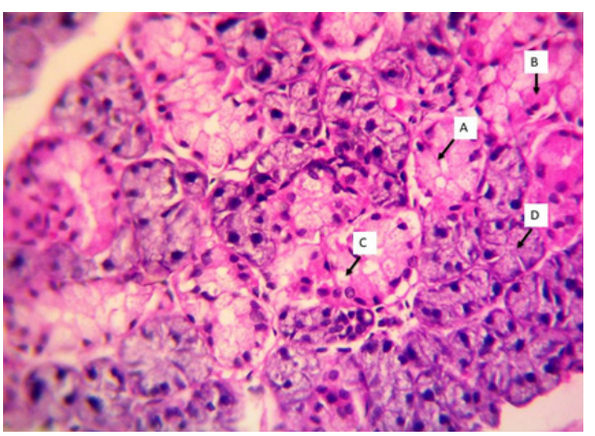

Fig. 10: A photomicrograph of a section of the submandibular salivary gland of a treatment group (Group V) shows most of the granular convoluted tubules returned to granular appearance (A) with an eosinophilic appearance (B) and some degeneration of a few of them (C) and normal serous acini (D). (H & E stain x 400).

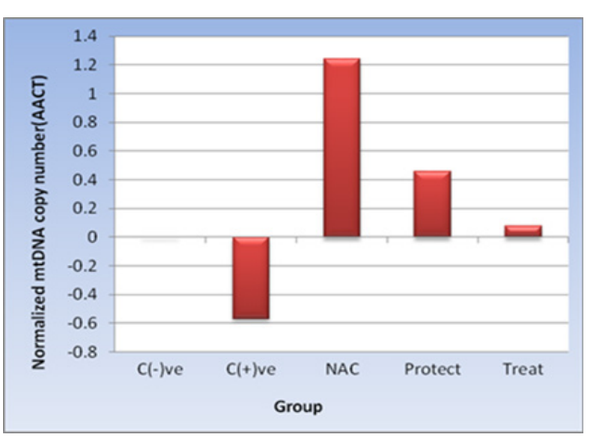

Fig. 11: mtDNA-CN in salivary glands' specimens of different groups of the study influenced by fructose over consumption and/or NAC treatment. mtDNA-CN was determined by q-PCR.

DISCUSSION

The current study investigated the detrimental effects of excessive fructose consumption on salivary glands and the protective role of NAC as an antioxidant that combats fructose's detrimental effects on these glands.

The link between chronically high fructose intake and increased energy intake, body weight, adiposity, hypertriglyceridemia, hyperlipidemia, hypertension, glucose intolerance, and decreased insulin sensitivity in laboratory animals is undeniable, resulting in MetS.[23]

To clarify the histological alterations, MetS has been linked to an inflammatory state. Inflammation may result in insulin resistance and oxidative stress, which may appear as MetS symptoms.[24] This could account for our observation of clogged (inflamed) blood vessels in the positive control group.

When the positive control group is compared to the negative control group, we observe an increase in eosinophilia of granular convoluted tubules. Our findings complemented previous research demonstrating a favorable connection between blood eosinophil counts and body mass index (BMI) or MetS.[25] Moussa *et al*.[26] identified a link between increased eosinophils in subcutaneous adipose tissue and MetS.

In 2011, Wu *et al*. demonstrated that increased eosinophils alleviated metabolic disturbances.[26] Numerous studies have demonstrated that eosinophils promote polarization of alternatively activated macrophages (AAM) in animal models.[27,28,29] These macrophages (AAMs/M2), in collaboration with group 2 innate lymphoid cells (ILC2s) and eosinophils, improve glucose tolerance and insulin sensitivity, as well as fat metabolism, thereby reducing obesity and its associated complications.[26]

The positive control group's lining epithelium atrophy is consistent with Lukach *et al*.[30] findings of a strong correlation between fatty replacement, fibrosis, and acinar atrophy, as well as between fatty replacement and hyperlipidemia. Klein *et al*.[31] demonstrated that fatty tissue replaces the parenchyma of the salivary glands, establishing for the first time a link between xerostomia symptoms, the metabolic/biologic process of hyperlipidemia, and pathological changes.

According to High *et al*., hypofunction of the submandibular gland is associated with an abnormal calcium signaling pathway.[32] This is also consistent with our observation of atrophy.

The presence of necrosis in the positive control group is consistent with the result of Matsumoto *et al*.[33], who demonstrated that hyperglycemic-induced oxidative stress damages saliva secretion-associated lipids and DNA. Hyperglycemia promotes the production of reactive oxygen species (ROS) and apoptotic and necrotic cell death.

Yamada *et al*.[34] demonstrated that supplementing with NAC, an exogenous antioxidant, inhibited ROS generation in a human salivary gland cell line in *vitro*. These findings were corroborated by the fact that NAC administration improved salivary secretion in a rat model of insulin resistance.[35] Taken together, these findings suggest that ROS plays a role in salivary gland injury. These conclusions can be used to interpret our findings in the study's protection and treatment groups.

Zalewska *et al*.[36] concluded that the effects of NAC supplementation in the salivary glands are dependent on the timing of its administration, i.e., the earlier NAC supplementation begins, the more satisfactory the effects.

Additionally, when rats were fed a high fat diet, NAC supplementation restored the activity of the mitochondrial respiratory chain complexes, decreased the ADP/ATP ratio, decreased ROS production, weakened apoptotic pathways, rescued the GSH pool, and prevented cytokine production to levels observed in control salivary glands[36] This enables us to interpret that the protection group's prognosis is superior and nearly identical to that of the negative control group, in contrast to the treatment group, which exhibits eosinophilia and irreversible degeneration and necrosis of some tubules.

Regarding the NAC group, it is very clear that this group is identical to the negative control group and undistinguishable. This will give us a clue that NAC did not affect the histomorphology of intact tissues either favorably or adversely.

In terms of gene expression, the positive control group exhibits a statistically significant decrease in mtDNA-CN expression when compared to the negative control group. According to Moreno-Fernández *et al*.[37], oxidative stress, which is primarily caused by mitochondrial dysfunction, is strongly associated with the development of MetS. Excessive energy supply results in increased oxidative activity, which, combined with insufficient antioxidant defense, results in an excess of ROS in mitochondria, resulting in damage to other macromolecules such as lipids, proteins, and nucleic acids.

A significant increase in mtDNA-CN has been observed in the NAC group. This is because NAC promotes glutathione biosynthesis, aids in detoxification, and acts directly as a free radical scavenger. It is a potent antioxidant and may be used to treat diseases associated with the production of free oxygen radicals.[38] This also fits Yamada *et al*. findings(mentioned above). Additionally, Mohammadi *et al*.[39] reported that NAC treatment significantly reduced mitochondrial permeability transition, enhanced mitochondrial membrane potential, and increased ATP levels.

Our finding demonstrates a significant elevation in mtDNA-CN of protection and treatment groups in relation to the negative control group. In addition, it is clear that the mtDNA-CN of the protection group is significantly superior to that of the treatment group. This gives us evidence that the opportunity for recovery is more

guaranteed in protection compared to the treatment group. Moreover, mtDNA-CN of the NAC group is maximal over all other groups. This will provide more protection against any prospective ROS-induced damage. It is obvious that all our findings regarding NAC, protection and treatment groups agree with Zalewska *et al*. (mentioned above).

CONCLUSION

Consumption of a high-fructose diet induced MetS and was proved to have harmful effects on the salivary gland. Moreover, this work provided a new insight into the possible use of NAC to alleviate the main features of MetS and to protect and cure the salivary glands from the damaging effects of fructose-induced MetS. It is important to know that the magnitude of NAC's effect is shown in a time-dependent manner.

ACKNOWLEDGEMENTS

We would like to express our gratitude and appreciation to the Department of Dental Basic Sciences at the College of Dentistry at the University of Mosul for their assistance in conducting this research.

ETHICS APPROVAL

This study followed the ethical committee's instructions and guidelines and received ethical approval no. (UoM. Dent/A.L.19/22).

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. El-Mehi, A. E. S., & Faried, M. A. (2020). Effect of high-fructose diet-induced metabolic syndrome on the pituitary-gonadal axis from adolescence through adulthood in male albino rats and the possible protective role of ginger extract. A biochemical, histological and immunohistochemical study. Folia morphologica, 79(4), 690-708. https://doi.org/10.5603/fm.a2019.0139.

2. Wong, W. Y., & Brown, L. (2014). Induction of metabolic syndrome by excess fructose consumption. In Diabetic Cardiomyopathy (pp. 41-63). Springer, New York, NY. http://dx.doi.org/10.1007/978-1-4614-9317-4_3.

3. Carresi, C., Gliozzi, M., Musolino, V., Scicchitano, M., Scarano, F., Bosco, F., ... & Mollace, V. (2020). The effect of natural antioxidants in the development of metabolic syndrome: Focus on bergamot polyphenolic fraction. Nutrients, 12(5), 1504. https://doi.org/10.3390/nu12051504.

4. Vona, R., Gambardella, L., Cittadini, C., Straface, E., & Pietraforte, D. (2019). Biomarkers of oxidative stress in metabolic syndrome and associated diseases. Oxidative medicine and cellular longevity, 2019. https://doi.org/10.1155/2019/8267234.

5. Monserrat-Mesquida, M., Quetglas-Llabrés, M., Capó, X., Bouzas, C., Mateos, D., Pons, A., ... & Sureda, A. (2020). Metabolic syndrome is associated with oxidative stress and proinflammatory state. Antioxidants,9(3), 236. https://doi.org/10.3390/antiox9030236.

6. Satoh, M., & Kuroiwa, T. (1991). Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. Experimental cell research, 196(1), 137-140. https://doi.org/10.1016/0014-4827(91)90467-9.

7. Lenart, J. (2017). Mitochondria in brain hypoxia. Postepy Higieny i Medycyny Doswiadczalnej (Online), 71, 118-128. https://doi.org/10.5604/01.3001.0010.3796.

8. Révész, D., Verhoeven, J. E., Picard, M., Lin, J., Sidney, S., Epel, E. S., ... & Puterman, E. (2018). Associations between cellular aging markers and metabolic syndrome: findings from the CARDIA study. The Journal of Clinical Endocrinology & Metabolism, 103(1), 148-157. https://doi.org/10.1210/jc.2017-01625.

9. Huang, C. H., Su, S. L., Hsieh, M. C., Cheng, W. L., Chang, C. C., Wu, H. L., ... & Liu, C. S. (2011). Depleted leukocyte mitochondrial DNA copy number in metabolic syndrome. Journal of atherosclerosis and thrombosis, 1108150409-1108150409. https://doi.org/10.5551/jat.8698.

10. Fazzini, F., Lamina, C., Raftopoulou, A., Koller, A., Fuchsberger, C., Pattaro, C. ... & GCKD Investigators. (2021). Association of mitochondrial DNA copy number with metabolic syndrome and type 2 diabetes in 14 176 individuals. Journal of Internal Medicine, 290(1), 190-202. https://doi.org/10.1111/joim.13242.

11. Salamonowicz, M. M., Zalewska, A., & Maciejczyk, M. (2019). Oral consequences of obesity and metabolic syndrome in children and adolescents. https://doi.org/10.17219/dmp/102620.

12. Bastin, A. J., Davies, N., Lim, E., Quinlan, G. J., & Griffiths, M. J. (2016). Systemic inflammation and oxidative stress post-lung resection: Effect of pretreatment with N-acetylcysteine. Respirology, 21(1), 180-187. https://doi.org/10.1111/resp.12662.

13. De Rosa, S. C., Zaretsky, M. D., Dubs, J. G., Roederer, M., Anderson, M., Green, A., ... & Herzenberg, L. A. (2000). N-acetylcysteine replenishes glutathione in HIV infection. European journal of clinical investigation, 30(10), 915-929. https://doi.org/10.1046/j.1365-2362.2000.00736.x.

14. Dludla, P. V., Dias, S. C., Obonye, N., Johnson, R., Louw, J., & Nkambule, B. B. (2018). A systematic review on the protective effect of N-acetyl cysteine against diabetes-associated cardiovascular complications. American Journal of Cardiovascular Drugs, 18(4), 283-298. https://doi.org/10.1007/s40256-018-0275-2.

15. Dludla, P. V., Orlando, P., Silvestri, S., Mazibuko-Mbeje, S. E., Johnson, R., Marcheggiani, F., ... & Tiano, L. (2019). N-Acetyl cysteine ameliorates hyperglycemia-induced cardiomyocyte toxicity by improving mitochondrial energetics and enhancing endogenous Coenzyme Q9/10 levels. Toxicology Reports, 6, 1240-1245. https://doi.org/10.1016/j.toxrep.2019.11.004 .

16. Bateman, D. N., & Dear, J. W. (2019). Acetylcysteine in paracetamol poisoning: a perspective of 45 years of use. Toxicology research, 8(4), 489-498. https://doi.org/10.1039/c9tx00002j.

17. Dludla, P. V., Nkambule, B. B., Mazibuko-Mbeje, S. E., Nyambuya, T. M., Marcheggiani, F., Cirilli, I., ... & Tiano, L. (2020). N-acetyl cysteine targets hepatic lipid accumulation to curb oxidative stress and inflammation in NAFLD: a comprehensive analysis of the literature. Antioxidants, 9(12), 1283. https://doi.org/10.3390/antiox9121283.

18. Di Luccia, B., Crescenzo, R., Mazzoli, A., Cigliano, L., Venditti, P., Walser, J. C., ... & Iossa, S. (2015). Rescue of fructose-induced metabolic syndrome by antibiotics or faecal transplantation in a rat model of obesity. PLoS One, 10(8), e0134893. https://doi.org/10.1371/journal.pone.0134893.

19. Crescenzo, R., Bianco, F., Coppola, P., Mazzoli, A., Valiante, S., Liverini, G., & Iossa, S. (2014). Adipose tissue remodeling in rats exhibiting fructose-induced obesity. European journal of nutrition, 53(2), 413-419. https://doi.org/10.1007/s00394-013-0538-2.

20. Breitbart, R., Abu-Kishk, I., Kozer, E., Ben-Assa, E., Goldstein, L. H., Youngster, I., & Berkovitch, M. (2011). Intraperitoneal N-acetylcysteine for acute iron intoxication in rats. Drug and Chemical Toxicology, 34(4), 429-432. https://doi.org/10.3109/01480545.2011.564176.

21. Hanci, V., Kerimoğlu, A., Koca, K., Başkesen, A., Kilic, K., & Taştekin, D. (2010). The biochemical effectiveness of N-acetylcysteine in experimental spinal cord injury in rats. Ulus Travma Acil Cerrahi Derg, 16(1), 15-21.

22. Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology.

23. Wong, S. K., Chin, K. Y., Suhaimi, F. H., Fairus, A., & Ima-Nirwana, S. (2016). Animal models of metabolic syndrome: a review. Nutrition & metabolism, 13(1), 1-12. https://doi.org/10.1186/s12986-016-0123-9.

24. Darroudi, S., Fereydouni, N., Tayefi, M., Ahmadnezhad, M., Zamani, P., Tayefi, B., ... & Ghayour-Mobarhan, M. (2019). Oxidative stress and inflammation, two features associated with a high percentage body fat, and that may lead to diabetes mellitus and metabolic syndrome. BioFactors, 45(1), 35-42. https://doi.org/10.1002/biof.1459.

25. Sunadome, H., Matsumoto, H., Izuhara, Y., Nagasaki, T., Kanemitsu, Y., Ishiyama, Y., ... & Hirai, T. (2020). Correlation between eosinophil count, its genetic background and body mass index: The Nagahama Study. Allergology International, 69(1), 46-52. https://doi.org/10.1016/j.alit.2019.05.012.

26. Moussa, K., Gurung, P., Adams-Huet, B., Devaraj, S., & Jialal, I. (2019). Increased eosinophils in adipose tissue of metabolic syndrome. Journal of diabetes and its complications, 33(8), 535-538. https://doi.org/10.1016/j.jdiacomp.2019.05.010.

27. Knights, A. J., Vohralik, E. J., Hoehn, K. L., Crossley, M., & Quinlan, K. G. (2018). Defining eosinophil function in adiposity and weight loss. Bioessays, 40(10), 1800098. https://doi.org/10.1002/bies.201800098.

28. Bolus, W. R., & Hasty, A. H. (2019). Contributions of innate type 2 inflammation to adipose function. Journal of Lipid Research, 60(10), 1698-1709. https://doi.org/10.1194/jlr.R085993.

29. Marichal, T., Mesnil, C., & Bureau, F. (2017). Homeostatic eosinophils: characteristics and functions. Frontiers in medicine, 4, 101. https://doi.org/10.3389/fmed.2017.00101.

30. Lukach, L., Maly, A., Zini, A., & Aframian, D. J. (2014). Morphometrical study of minor salivary gland in xerostomic patients with altered lipid metabolism. Oral diseases, 20(7), 714-719. https://doi.org/10.1111/odi.12195.

31. Klein, A., Klein, J., Chacham, M., Kleinman, S., Shuster, A., Peleg, O., ... & Kaplan, I. (2022). Acinar Atrophy, Fibrosis and Fatty Changes Are Significantly More Common than Sjogren's Syndrome in Minor Salivary Gland Biopsies. Medicina, 58(2), 175. https://doi.org/10.3390/medicina58020175.

32. Chen, S. Y., Wang, Y., Zhang, C. L., & Yang, Z. M. (2020). Decreased basal and stimulated salivary parameters by histopathological lesions and secretory dysfunction of parotid and submandibular glands in rats with type 2 diabetes. Experimental and Therapeutic Medicine, 19(4), 2707-2719. https://doi.org/10.3892/etm.2020.8505.

33. Matsumoto, N., Omagari, D., Ushikoshi-Nakayama, R., Yamazaki, T., Inoue, H., & Saito, I. (2021). Hyperglycemia induces generation of reactive oxygen species and accelerates apoptotic cell death in salivary gland cells. Pathobiology, 88(3), 234-241. https://doi.org/10.1159/000512639.

34. Yamada, T., Ryo, K., Tai, Y., Tamaki, Y., Inoue, H., Mishima, K., ... & Saito, I. (2010). Evaluation of therapeutic effects of astaxanthin on impairments in salivary secretion. Journal of Clinical Biochemistry and Nutrition, 1006210038-1006210038. https://doi.org/10.3164/jcbn.10-31.

35. Żukowski, P., Maciejczyk, M., Matczuk, J., Kurek, K., Waszkiel, D., Żendzian-Piotrowska, M., & Zalewska, A. (2018). Effect of N-acetylcysteine on antioxidant defense, oxidative modification, and salivary gland function in a rat model of insulin resistance. Oxidative Medicine and Cellular Longevity, 2018. https://doi.org/10.1155/2018/6581970.

36. Zalewska, A., Szarmach, I., Żendzian-Piotrowska, M., & Maciejczyk, M. (2020). The effect of N-acetylcysteine on respiratory enzymes, ADP/ATP ratio, glutathione metabolism, and nitrosative stress in the salivary gland mitochondria of insulin resistant rats. Nutrients, 12(2), 458. https://doi.org/10.3390/nu12020458.

37. Moreno-Fernández, S., Garcés-Rimón, M., Vera, G., Astier, J., Landrier, J. F., & Miguel, M. (2018). High fat/high glucose diet induces metabolic syndrome in an experimental rat model. Nutrients, 10(10), 1502. https://doi.org/10.3390/nu10101502.

38. Xie, C., Yi, J., Lu, J., Nie, M., Huang, M., Rong, J., ... & Shu, X. (2018). N-acetylcysteine reduces ROS-mediated oxidative DNA damage and PI3K/Akt pathway activation induced by helicobacter pylori infection. Oxidative Medicine and Cellular Longevity, 2018. https://doi.org/10.1155/2018/1874985.

39. Mohammadi, H., Sayad, A., Mohammadi, M., Niknahad, H., & Heidari, R. (2020). N-acetyl cysteine treatment preserves mitochondrial indices of functionality in the brain of hyperammonemic mice. Clinical and Experimental Hepatology, 6(2), 106. https://dx.doi.org/10.5114%2Fceh.2020.95814.

الملخص العربى

الدراسة الجزيئية والنسيجية لـتأثير N-acetyl cysteine على الغدد اللعابية في متلازمة التمثيل الغذائي التي يسببها الفركتوز في الجرذان البيضاء

[١]اوس زكريا يحيى، [٢]غادة عبد الرحمن طاقة، [٣]محمد عبد الغفور القطان

[١]وزارة الصحة، دائرة صحة نينوى، الموصل، العراق

[٢]قسم العلوم الأساسية لطب الأسنان، كلية طب الأسنان، جامعة الموصل، الموصل، العراق

[٣]قسم الكيمياء الحيوية، كلية الطب، جامعة نينوى، الموصل، العراق

مقدمه: يمكن أن يؤدي اتباع نظام غذائي غني بالفركتوز إلى متلازمة التمثيل الغذائي. يحدث MetS نتيجة لمجموعة من المتغيرات الوراثية والمكتسبة التي تسبب الإجهاد التأكسدي ، والخلل الخلوي ، والالتهاب الجهازي. N-acetylcysteine (NAC)) هو المعيار الذهبي لعلاج سمية الباراسيتامول. بالإضافة إلى ذلك ، أصبح NAC نموذجًا مثاليًا لـ "مضادات الأكسدة" على مدار الوقت. يستخدم غالبية الباحثين ويقيمون NAC على أمل منع أو تخفيف الإجهاد التأكسدي.

الهدف: لتقييم التأثيرات المحسّنة المحتملة لـ NAC على الغدد اللعابية من MetS المستحثة بالفركتوز في الجرذان.

المواد والطرق: تم تقسيم أربعين ذكرًا من الجرذان البيضاء، تتراوح أعمارهم بين ١٠-١٢ أسبوعًا، بشكل عشوائي إلى خمس مجموعات متساوية. تلقت المجموعة الأولى (التحكم السلبي) ماء الصنبور لمدة ١٢ أسبوعًا. المجموعة الثانية (التحكم الإيجابي) تلقت ٦٠٪ وزن / وزن شراب الفركتوز بدلاً من ماء الصنبور لمدة ١٢ أسبوعًا. تلقت المجموعة الثالثة (NAC) ماء الصنبور وحقنة داخل البريتون (IP) من NAC (١٥٠ مجم / كجم / يوم) لمدة ١٢ أسبوعًا. تلقت المجموعة الرابعة (الحماية) ٦٠ ٪ FS عن طريق الفم في نفس الوقت مع حقن NAC IP (١٥٠ مجم / كجم / يوم) لمدة ١٢ أسبوعًا. تلقت المجموعة الخامسة (العلاج) ٦٠٪ FS لمدة ٨ أسابيع تليها ٤ أسابيع من شرب ماء الصنبور مع حقن NAC IP (١٥٠ مجم / كجم / يوم). تم قتل الجرذان في نهاية ١٢ أسبوعا. تم استئصال الغدد اللعابية وفحصها تشريحياً ولعدد نسخ الحمض النووي للميتوكوندريا (mtDNA-CN).

النتائج: من الناحية النسيجية ، أظهرت المجموعة الثانية فرط الحمضات والضمور والنخر. عرضت المجموعة الثالثة صورة معمارية عادية. في المجموعة الرابعة ، كانت هناك صورة معمارية طبيعية مع تنكس ظهاري خفيف. في المجموعة الخامسة ، عادت معظم الأنابيب إلى مظهرها الغدي ، ولكن لا يزال هناك فرط الحمضات وبعض التنكس والنخر. جزيئيًا ، في المجموعة الثانية ، كان هناك انخفاض كبير في mtDNA-CN. وأظهرت المجموعة الثالثة أعلى نسبة من mtDNA-CN بين جميع المجموعات. في المجموعة الرابعة ، لوحظت زيادة كبيرة في mtDNA-CN ، ولكن أقل من المجموعة الثالثة. أظهرت المجموعة الخامسة أيضًا زيادة كبيرة ، ولكن أقل من المجموعة الرابعة.

الخلاصة: الأنظمة الغذائية عالية الفركتوز تحفز متلازمة الأيض وثبت أنها تضر بالغدد اللعابية. من ناحية أخرى ، ثبت أن NAC يخفف من MetS ويحمي ويعالج الغدد اللعابية من التأثيرات الضارة لـ MetS بطريقة تعتمد على الوقت.