Prophylactic Effect of N-Acetyl Cysteine on Salivary Glands Against Oxidative Stress in Male Albino Rats: Biochemical and Histological Study

Original Article

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ABSTRACT

Aim of the Study: This research aimed to examine the protective antioxidant role of N-acetyl cysteine (NAC) against H2O2 induced oxidative stress in male albino rats.

Material and Methods: Forty adult male albino rats were randomly divided into 4 groups: Group1 (Negative Control Group) (n=10): Normal diet and tap water for drinking, injected daily with 1ml/kg distilled water(DW) (i.p.) for 4 weeks. Group2: (H2O2 Treated Group) (n=10) received normal diet, drinking water containing 0.5% H2O2 daily to induce oxidative stress and injected daily with 1 ml/kg body weight distilled water(i.p.) for 4 weeks. Group3: (NAC Group) (n=10) received normal diet, tap water for drinking and injected daily with NAC 150 mg /kg body weight (i. p.) for 4 weeks. Group 4: (Protected Group) (NAC+H2O2) (n=10) received normal diet and drinking water contain 0.5% H202 daily to induce oxidative stress and injected daily with NAC 150 mg. /kg body weight (i. p.) for 4 weeks. Blood samples were collected from all the animals groups for testing the antioxidant enzymes (Catalase and Glutathione peroxidase) and blood glucose level. The body weights were checked. At end of the experiment the animals were anaesthetized. The salivary glands were dissected for histologic investigation.

Results: Showed a significant reduction in levels of both Catalase and Glutathione peroxidase enzymes and a significant increase in the blood glucose level at $p \leq 0.05$, and a severs degeneration in salivary glands tissue for the H2O2 Treated Group compared to the Negative Control Group, NAC Group and Protected Group (NAC+H2O2) which show no significant changes.

Conclusion: This study indicates the prophylactic and protective roles of NAC against H2O2 induced oxidative stress deteriorating effect on salivary glands tissue, antioxidant enzymes, and blood glucose level in male albino rats.

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Key Words: Antioxidant; N-acetyl cysteine; oxidative stress; salivary glands.

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INTRODUCTION

Oxidative stress is an imbalance between the generation of reactive oxygen species (ROS) and the ability of cells to neutralize them by antioxidant systems which lead to an excess level of ROS[1]. ROS are active biomolecules that physiologically result as by production of metabolic pathways and/or by immune cells. ROS low levels are physiologically important for many molecular pathways such as cellular signaling, defense mechanism against invading pathogens, for proper blood flow, important for normal neuron activity, and normal growth and apoptosis[2,3]. When ROS exceed the physiological level, it overcomes the antioxidant capacity of cells leading to an oxidative stress which plays a central role in the pathophysiology of many diseases and health defects[4]. Excessive ROS leads to biomolecules and DNA strands damage, excessive peroxidation of lipid[5]. ROS also impairs gene expression, cytokine production, cellular metabolism change signaling pathways, promoting cellular injury, neuronal death, and mitochondrial dysfunction in addition to dysfunction of receptors, ion channels, and other membrane proteins leading to impair fluidity and permeability of the cell membrane[6]. To avoid oxidative damages, the antioxidant compounds act as a defense system. The antioxidants are divided to enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants is catalyzing the break down and removal of ROS by conversion of the oxidative products into hydrogen peroxide. Hydrogen peroxide is then converted to water in the presence of cofactor metals as copper, zinc, manganese and iron. These enzymatic antioxidants maintain the structure and function of the cell membrane by preventing lipid peroxidation, examples include: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx). Non-enzymatic antioxidants function is interruption of free radicals chain reaction (examples: vitamin C, vitamin E, plant polyphenol, carotenoids and glutathione[7,8]. N-acetyl cysteine (NAC):

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NAC is widely used as an antioxidant, clinically it is used as a mucolytic agent for many respiratory diseases and to treat acetaminophen overdose. NAC is a thiol compound and acts as an acetylated precursor of the amino acid L-cysteine which is a building block of glutathione (GSH)[9]. The NAC direct antioxidant activity is due its free thiol group that reacts with reactive oxygen (ROS) and act as scavenger of them, while the indirect antioxidant activity is related to the Cysteine which is the building block of glutathione[10]. GSH is the most available non protein thiol in the body and the powerful antioxidant responsible for maintaining the cellular redox status which reacts directly with reactive species, and acts as a cofactor or substrate for antioxidant enzymes[11]. This study objected to investigate NAC prophylactic role on antioxidant enzymes, blood glucose level, body weight and salivary gland tissue against H_2O_2 induced oxidative stress.

MATERIAL AND METHODS

Experimental models

This study was performed in the Pharmacology Laboratory at the Dental Basic Science Department, College of Dentistry, University of Mosul- Iraq. Forty Albino male rats were kept in separated plastic cages with wood shavings bed, free access to a standard diet and water. The rats' ages were 10-12 weeks and their weight 190-220 gm. A standard housing conditions were kept for the rats: light/ dark cycle 12-hours light/ 12 hours dark, temperature 20-21°C, humidity according to the external environmental conditions[12]. The animals checked by Veterinary Physician daily. This study was done according to the guidelines and approval of the Ethical Committee.no (UoM.Dent/A.L.8/22).

Medications

Acetyl cysteine (NAC): ampule 300 mg/3 ml. (Asist/ Bilim®, Turkey). Dose of NAC in this study was 150 mg/ kg body weight injected intraperitoneally (i.p)[13]., daily for 4weeks[12] Hydrogen peroxide(H_2O_2):solution extra pure (Scharlau®,Spain). This solution was diluted with distilled water to prepare 0.5% H_2O_2 working solution daily and used as drinking water for induction of oxidative stress for 4 weeks[14].

Study Design

Animals were divided into 4 groups: **Group1:** (Negative Control Group) (10 rats): received normal diet and tap water for drinking and injected daily with 1ml /kg body weight distilled water intraperitoneally for 4 weeks. **Group 2:** (H_2O_2 Treated Group) (10 rats) received normal diet and drink water containing 0.5% H_2O_2 daily to induce oxidative stress. injected daily with 1 ml/kg body weight distilled water intraperitoneally for 4 weeks. **Group3:** (NAC Group) (10 rats) received normal diet and tap water for drinking and injected daily with NAC 150 mg. /kg body weight intraperitoneally for 4 weeks. Group4:(Protected Group)(NAC+H2O2) (10 rats) received normal diet and drinking water containing 0.5% H_2O_2 daily to induce oxidative stress and injected daily with NAC150 mg./kg body weight intraperitoneally for 4 weeks.

Body Weight

All animals were weighted at day 0, 1st week, 2nd week, 3rd week, and 4th week of experiment. Dose of NAC was tapered according to the body weight and to evaluate effect of the oxidative stress and NAC on the body weight.

Blood samples collection

Blood samples were collected from all the animals groups at day 0 of experiment and after 4th week from the orbital plexus vein of the rats[15]. Blood samples collected in gel tubes, kept at room temperature for 15 minutes. Then the serum was separated by putting blood samples in centrifuges 3500 rpm for 15 minutes and then put in a separated Eppendrof and put in deep freeze (-20 °C) until used for assay of the serum catalase and glutathione peroxidase enzyme.

Biochemical Assay

Assay of the Blood Glucose

The blood glucose was tested from the tail using glucometer (Viva Check)® for all the animal groups This assay was done at day 0, 2nd week and 4th week of experiment.

Assay of the serum glutathione peroxidase

This test was done by using Rat GSH-Px ELISA Kit (Nanjing Duly Biotech Co., Ltd China) For the quantitative in *vitro* determination of Rat Glutathione peroxidase concentrations in serum by ELISA . Serum glutathione peroxidase was measured for all animals group at day 0 and after 4 weeks at end of experiment.

Assay of the serum Catalase

This test was done by using Catalase (CAT) Activity Assay Kit (Elabscience)®. The serum catalase enzyme was measured for all the animal groups at day 0 and after 4 weeks at end of the experiment.

Tissues collection and Histological Investigation

After 4 weeks of the experiment, all the animal groups were anaesthetized and salivary glands were extracted, Species of salivary glands for each animal were kept in 10% formaldehyde (fixation). The histological investigation was done by passing in 4 steps[16]; dehydration, clearing, impregnation, embedding, Sectioning and staining by Hematoxylin and Eosin stain (H & E). The light microscope evaluation was performed by 2 pathological specialists for investigation of the results by using a digital camera which is connected to the microscope.

Statistical analysis

Statistical Analysis was performed by SPSS program version 21 for Windows software. Descriptive statistics of data were expressed as (mean ± standard deviation) values.

The differences between the 4 groups were statistically analyzed by one-way analysis of variance (ANOVA) with post-hoc Duncan's test for data of body weight, biochemical test data. The differences were considered significant at $p \leq 0.05$.

RESULTS

Body weight

The body weight data revealed no change in growth scale during the 4 weeks of the experiment for control positive group (H_2O_2) compared to each of Negative Control, Protected Group (NAC+H_2O_2) and NAC Group (Figure 1).

Blood Glucose Level

Data of this work revealed no significant difference between values of the Negative Control Group at day zero, week 2 and week 4 (112.33±12.42) , (110.33 ±4.93) and (109.66 ± 3.05) respectively. For H_2O_2 Treated Group, there is a statistical significant increase in the values at week 2 and week 4, (119.4 ± 12.44) (134.6 ±7.44) respectively compared to day0 (111.8 ±11.32). For the Protected Group (H_2O_2+NAC), there is no statistical significant difference between the values at day zero, week 2 and week 4 (111.4±11.41) , (112.2±9.50) and (113±7.87) respectively. For NAC group no statistical significant difference between values at day zero, week 2 and week 4 (112.2±14.04), (110.8±12.87) and (111.6 ± 10.01) respectively. Statistical analysis by ANOVA-test revealed a statistically significant increase of values of H_2O_2 Treated Group compared to each of the Negative Control, (H_2O_2+NAC) and NAC Group at week 4. (Figure 2).

Catalase (CAT)

Data of this work revealed no statistically significant difference between values of control negative group at day zero and week 4 (30.22±9.75) (28.93±7.48) respectively. There is a statistically significant difference between the values of H_2O_2 Treated Group at day zero and week 4 (33.9±2.42) (16.14 ±9.48) respectively due to oxidative effect of H_2O_2 which lead to reduction of value at week 4. No statistical significant difference between values of NAC group at day zero and week4 (28.44±8.89)(27.02±2.43) respectively. Statistical analysis by ANOVA-test revealed statistical significant difference between values of H_2O_2 Treated Group from each of Negative Control, (H_2O_2+NAC) and NAC group at week4. (Figure 3)

GPx

Data of this work revealed no statistical significant difference between the values of the Negative Control Group at day zero and week 4 (2.5±0.66) (2.63±0.056) respectively. There is statistical significant difference between values of H_2O_2 Treated Group at day zero and week 4 (2.5±0.66) (0.75±0. 21) respectively due to oxidative effect of H_2O_2 which lead to reduction of value at week 4. No statistical significant difference between the values of (H_2O_2+NAC) Group at day zero and week 4 (2.5±0.66) (2.37±0.31) respectively. No statistical significant difference between values of NAC group at day zero and week4 (2.5±0.66) (2.47±0.74) respectively. Statistical analysis by ANOVA-test revealed statistical significant difference between the values of H_2O_2 Treated Group from each of the Negative Control, (H_2O_2+NAC) and (NAC) groups at week 4. (Figure 4)

Histological Results

The microscopic examination of the salivary glands sections showed that Negative Control and NAC groups had no histological abnormalities and had intact acini and ducts, regular cells of salivary glands (Figure 5,6). The H_2O_2 Treated Group had many pathological changes as a severe necrosis of acini, striated duct and granular convoluted tubules, increase of fibrous tissue that surround interlobular ducts, edema between lobules and congestion of blood vessels (Figure 7). The Protected Group (H_2O_2+NAC) had intact mucous, serous acini and interlobular ducts with the damage of the epithelium lining of the granular convoluted tubules (Figure 8).

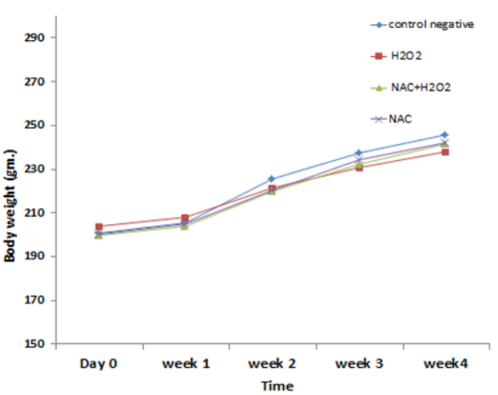

Fig. 1: Body weight data, the values are expressed as (mean ± standard deviation)

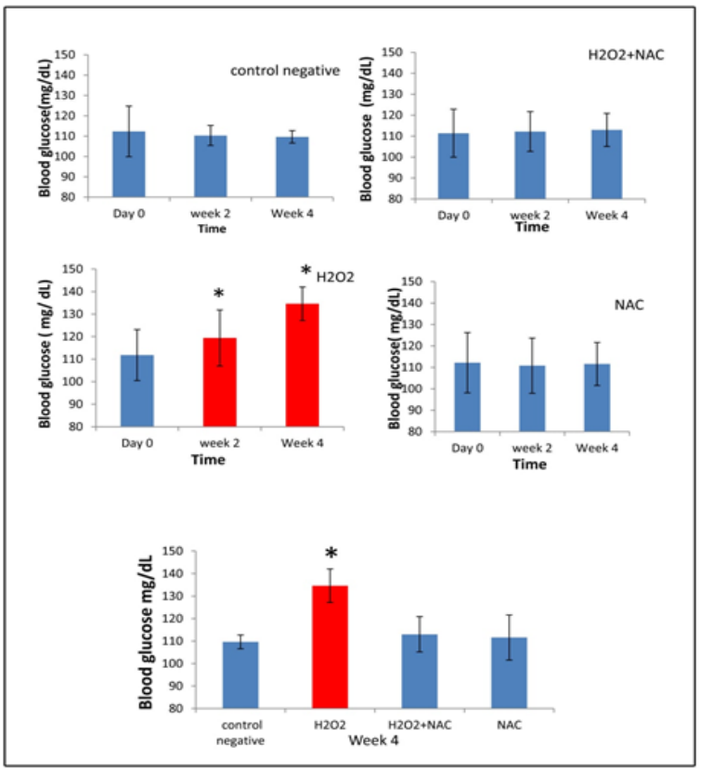

Fig. 2: Blood glucose data, the values are expressed as(mean ± standard deviation). * indicates a statistically significant difference at $p \leq 0.05$

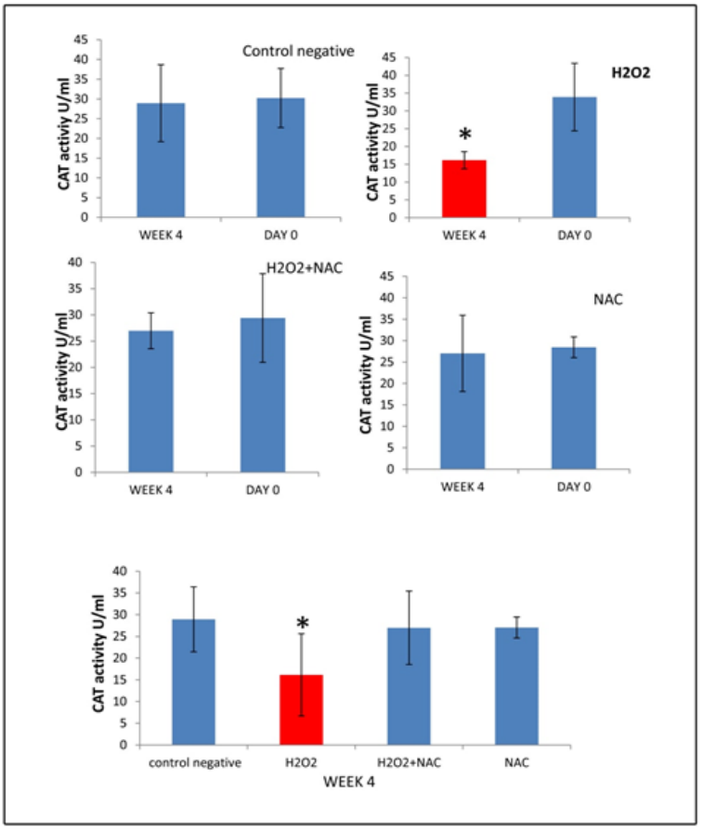

Fig. 3: Serum CAT data values are expressed as(mean ± standard deviation). * indicates a statistically significant difference at $p \leq 0.05$

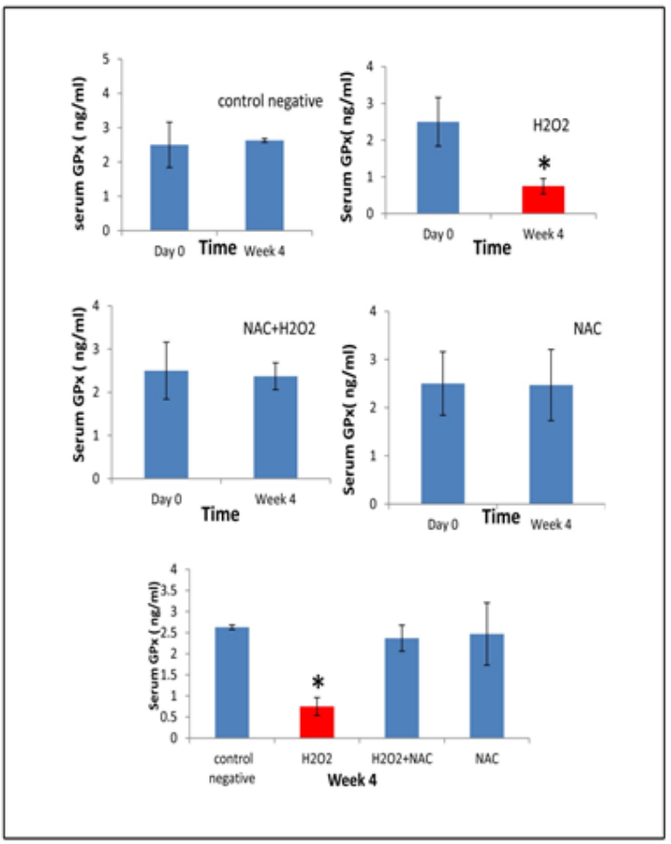

Fig. 4: Serum GPx data values are expressed as(mean ± standard deviation). * indicates a statistically significant difference at $p \leq 0.05$

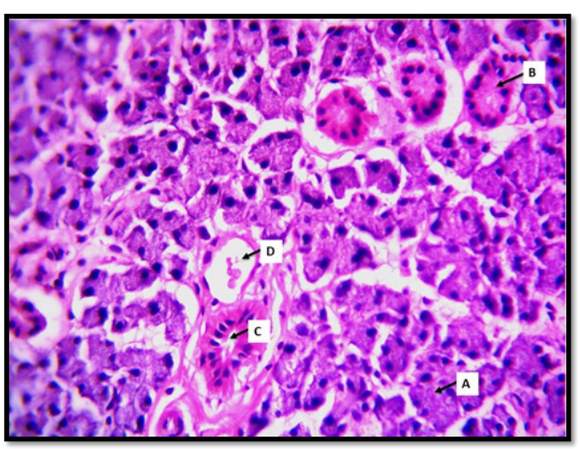

Fig. 5: photomicrograph of rat salivary gland of control negative Group shows intact mucous and serous acini (A), granular convoluted tubules (B), straight ducts (C) and blood vessel (D). H and E stain ,X 400

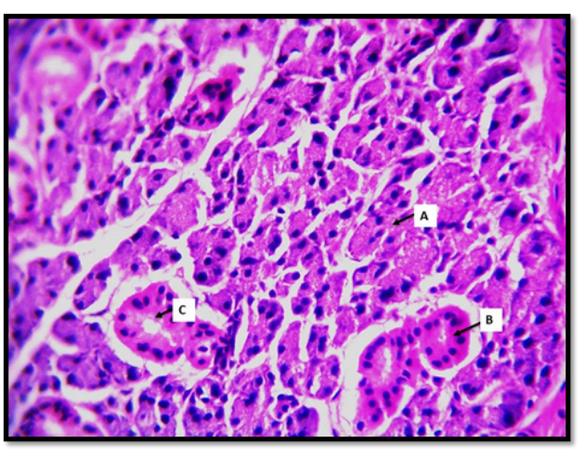

Fig. 6: photomicrograph of rat salivary gland of NAC Group shows intact mucous and serous acini (A), granular convoluted tubules (B), straight ducts (C). H and E stain ,X 400

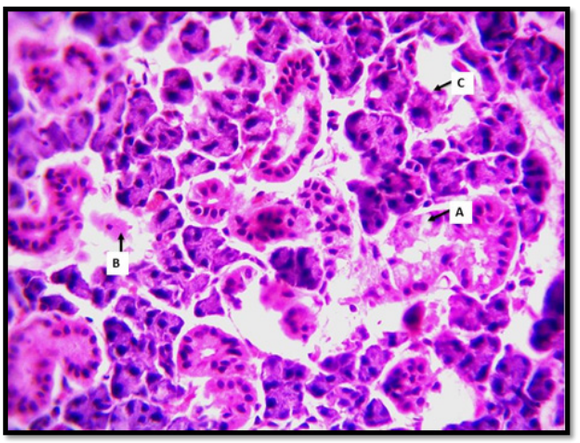

Fig. 7: photomicrograph of rat salivary gland of H2O2 Treated Group shows severe degeneration (A) and necrosis with atrophy (B) of epithelial cells lining granular convoluted tubules and atrophy of acini (C). H and E stain ,X 400

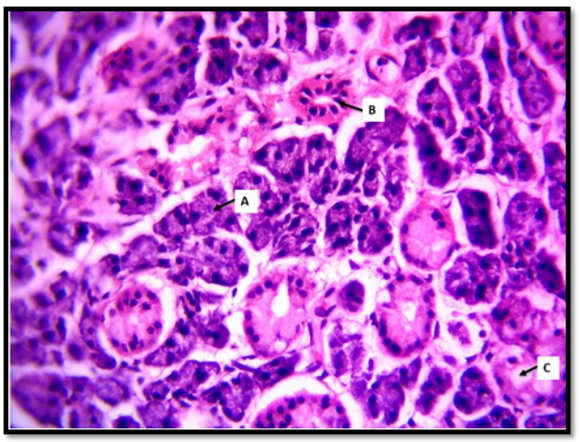

Fig. 8: photomicrograph of rat salivary gland of Protected Group(H2O2 + NAC) shows intact mucous and serous acini (A) and interlobular ducts (B) with degeneration of epithelium lining granular convoluted tubules (C). H and E stain ,X 400

DISCUSSION

Body weight

No change in the growth scale during 4 weeks of the experiment for the Positive Control Group (H_2O_2) compared to each of the Negative Control, Protected ($NAC+H_2O_2$) and NAC groups. This result disagree with another study that showed supression of growth scale for H_2O_2 Treated Group due to the toxicity of H_2O_2[17]. This can be explained by suggestion of Bouayed and Soulimani , 2019 who suggest that acute exposure to H_2O_2 oxidative stress exclude change of body weight due to oxidative stress induced anxiety and metabolic dysfunction[18].

CAT and GPx

The production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and the removal of reactive oxygen species in organisms is in a dynamic balance in the normal condition. When the ROS exceeds the physiological limit, this balance is broken, the biological macromolecules will be damaged[19]. The antioxidant defensing enzyme system such as CAT and GPx are investigated in this study. CAT convert H_2O_2 into H_2O and O_2, glutathione peroxidase (GPx) function is the breakdown of H_2O_2 and oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG), these enzymes lead to protection of the cells from DNA damage and lipid peroxidation maintaining the cell integrity[20]. The results of this study showed a significant reduction in serum CAT and GPx activity in the H_2O_2 Treated Group after 4 weeks of administration H_2O_2 compared to day 0, which indicate oxidative stress state. H_2O_2 is a powerful oxidizing agent that leads to a depletion of GSH and a decrease NADPH production via an inhibition of Pentose phosphate shunt. NADPH is necessary to activate the enzyme glutathione reductase which it is required to re-manufacture of GSH from oxidized GSH[21]. NADPH is important for maintaining normal activity of antioxidant enzymes CAT and GPx for biosynthesis reaction, ATP production and protection against ROS damage[22]. H_2O_2 inhibits Nrf 2 and this also explain the reduction of CAT and GSH-Px activity by H_2O_2 because Nrf 2 is a transcription factor that regulates the expression of antioxidant enzymes, activation of Nrf2 reduce ROS production and improve the ability of protection against oxidative stress[23]. This result agree with other studies[24,25]. The Negative Control and NAC groups showed no significant changes between day 0 and week 4 data. Protected group (H_2O_2+NAC) showed no significant change in activity of both CAT and GPx at week 4 compared to day 0 indicate NAC powerful antioxidant capacity, while ANOVA analysis of the data at week 4 showed a significant difference of the H_2O_2Treated Group compared to Negative Control, NAC and Protective groups. NAC prevent oxidative stress because it acts as scavenger of ROS, precursor of GSH and NAC treatment lead to increase the production of endogenous H2S which inhibit apoptosis, enhance mitochondrial bioenergetics and protect against 'oxidative stress[26]. These results agree with that of the studies of[27,28].

Blood Glucose Level

Blood glucose was tested for all groups at day 0,2nd week and 4th week. Results for H_2O_2 treated group showed a significant increase of the blood glucose values at 2nd week and 4th week compared to day 0 value for the same group and compared to the Negative Control, Protected (H_2O_2+NAC) and NAC groups. Oxidative stress and hyperglycemia are cross-linked. ROS as H_2O_2 aggravates the stress signaling pathways in β-cells of the pancreas as a result of activation of (NF- κB) which causes apoptosis and their damage. Oxidative stress also inhibits the respiratory chain in β-cells and reduces ATP generation that leads to suppression of the glucose-mediated insulin secretion and elevation of the blood glucose level[29]. This result is in agreement with the results of other studies[30,31]. The results showed that no significant changes in the blood glucose

level for each of NAC and (NAC+H_2O_2) groups at 2nd week and 4th week compared to the values at day 0 also compared to the Negative Control Group. Results indicate NAC has beneficial effect on the blood glucose level. This can be explained by the antioxidant role of NAC as a scavenger of free radical, glutathione precursor and activator of antioxidant enzymes so preventing disturbance of blood glucose induced by oxidative stress. Also, NAC improves mitochondrial glucose metabolism, improves sensitivity to insulin and prevents insulin resistant[32,33]. These results agree with the results of another study[34].

Histology

Oxidative stress leads to dysfunction of rats salivary glands and salivary secretion, Saliva has essential role in maintaining good health, for oral cavity and also for whole body by controlling homeostasis, moisturizing effect and cleaning of mucous membranes and teeth. Saliva start first stage of carbohydrate digestion and facilitates the swallowing of food. Saliva acts as buffering agent that maintain constant pH, protecting teeth against erosion and decay. Damage of salivary gland by oxidative stress lead to disruption of all these function[35].

ROS stimulates nuclear kappa B factor (NF-κB); a redox-sensitive transcription factor that leads to production of further inflammatory mediators[36]. H_2O_2 causes disruption of the plasma membrane and leads to necrosis[37]. This results was compatible with the histological results which showed necrosis, edema and congestion in blood vessels in the salivary gland of H_2O_2 Treated Group compared to Negative Control group. Damage of salivary glands by oxidative stress agreed with that of another study[38]. Protected group with NAC (NAC+H_2O_2) showed much fewer of degeneration changes compared to the Positive Control Group. This indicates the antioxidant and anti-inflammatory effect of NAC. N-Acetyl cysteine suppresses NF-κB by removal of ROS, NAC also inhibited the synthesis of other inflammatory cytokines as IL-1β, IL-6, IL-8, and TNFα[39]. Another mechanism related to the indirect antioxidant activity of NAC is related to its reducing capacity by restoring systemic stores of GSH and reduced protein sulfhydryl groups, which are involved in the regulation of the redox state[40].

CONCLUSION

N-Acetyl cysteine has prophylactic and protective antioxidant roles on the salivary glands tissue injury, (and antioxidant enzymes disturbance,) caused by H_2O_2 induced oxidative stress.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Szyguła-Jurkiewicz, B., Szczurek-Wasilewicz, W., Osadnik, T., Frycz-Kurek, A. M., Macioł-Skurk, K., Małyszek-Tumidajewicz, J. and Jóźwiak, J. J. (2022).Oxidative Stress Markers in Hypertrophic Cardiomyopathy Medicina, 58(1):31 https://doi.org/10.3390/medicina58010031.
2. Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V. and Bitto, A. (2017). Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity, https://doi.org/10.1155/2017/8416763
3. Milkovic, L., Cipak Gasparovic, A., Cindric, M., Mouthuy, P. A. and Zarkovic, N. (2019). Short overview of ROS as cell function regulators and their implications in therapy concepts. Cells, 8(8): 793, https://doi.org/10.3390/cells8080793
4. Morvaridzadeh, M., Estêvão, M. D., Qorbani, M., Heydari, H., sadat Hosseini, A., Fazelian, S. and Heshmati, J. (2022). The effect of chromium intake on oxidative stress parameters: A systematic review and meta-analysis. Journal of Trace Elements in Medicine and Biology, 69, 126879.
5. Ma, Y., Lee, G., Heo, S. Y., and Roh, Y. S. (2022). Oxidative Stress Is a Key Modulator in the Development of Nonalcoholic Fatty Liver Disease. Antioxidants, 11(1): 91, https://doi.org/10.3390/antiox11010091
6. Maciejczyk, M., Bielas, M., Zalewska, A., and Gerreth, K. (2021). Salivary Biomarkers of Oxidative Stress and Inflammation in Stroke Patients: From Basic Research to Clinical Practice. Oxidative Medicine and Cellular Longevity, 2021; 1-22 https://doi.org/10.1155/2021/5545330
7. Mitra, A. K. (2020). Antioxidants: a masterpiece of mother nature to prevent illness. Journal of Chemical Reviews, 2(4): 243-256.
8. Liu, P., Li, Y., Wang, R., Ren, F., & Wang, X. (2022). Oxidative Stress and Antioxidant Nanotherapeutic Approaches for Inflammatory Bowel Disease Biomedicines, 10(1):85,https://doi.org/10.3390/biomedicines10010085
9. Liao, C. Y., Wu, T. C., Yang, S. F., & Chang, J. T. (2022). Effects of NAC and Gallic Acid on the Proliferation Inhibition and Induced Death of Lung Cancer Cells with Different Antioxidant Capacities. Molecules, 27(1): 75, https://doi.org/10.3390/molecules27010075.
10. Altomare, A., Baron, G., Brioschi, M., Longoni, M., Butti, R., Valvassori, E .and Aldini, G. (2020). N-acetyl-cysteine regenerates albumin cys34 by a thiol-disulfide breaking mechanism: An explanation of its extracellular antioxidant activity. Antioxidants, 9(5):367, https://doi.org/10.3390/antiox9050367

11. Yamamoto, H., Shibuya, K., Fukushima, T. and Hashizume, T. (2022). Effects of antioxidant capacity on micronucleus induction by cigarette smoke in mammalian cells. Mutation Research/ Genetic Toxicology and Environmental Mutagenesis, 873: 503427, https://doi.org/10.1016/j.mrgentox.2021.503427

12. Zalewska, A., Zięba, S., Kostecka-Sochoń, P., Kossakowska, A., Żendzian-Piotrowska, M., Matczuk, J. and Maciejczyk, M. (2020). NAC Supplementation of Hyperglycemic Rats Prevents the Development of Insulin Resistance and Improves Antioxidant Status but Only Alleviates General and Salivary Gland Oxidative Stress. Oxidative Medicine and Cellular Longevity. https://doi.org/10.1155/2020/8831855.

13. Finamor, I. A., Ourique, G. M., Pês, T. S., Saccol, E. M., Bressan, C. A., Scheid, T., ... & Pavanato, M. A. (2014). The protective effect of N-acetylcysteine on oxidative stress in the brain caused by the long-term intake of aspartame by rats. Neurochemical research, 39(9): 1681-1690

14. Hameed, S., Abdlrhmaan, A. J., and Hameed, I. A. (2021). Effect of Cucurbita pepo L Extract on Histology Changes of Some Entry Organs Anatomy in the Males of Rats Exposed to Oxidative Stress. Clin Schizophr Relat Psychoses.15: S6,Doi: 10.3371/CSRP.HSAA.102821

15. Obi, P. E., Ezeorah, C. C., Odoh, U. E., and Offiah, R. O. (2022). Effect of ethanol leaf extract of Clerodendrum splendens (G. Don) (Verbenaceae) on some biochemical parameters of Alloxan-induced diabetic Wistar rats. Phytomedicine Plus, 2(1): 00147.

16. Luna, L.G. (1968). Manual of Histologic Staining Method of the Armed Forces Institute of Pathology. 3rd Edition. MaGraw- Hill Co., USA. 3-158. https://doi.org/10.1016/s0031-3025(16)39410-7.

17. Guesmi, F., Beghalem, H., Tyagi, A. K., Ali, M. B., Mouhoub, R. B., Bellamine, H., and Landoulsi, A. (2016). Prevention of H2O2 induced oxidative damages of rat testis by Thymus algeriensis. Biomedical and Environmental Sciences, 29(4): 275-285.

18. Bouayed, J., & Soulimani, R. (2019). Evidence that hydrogen peroxide, a component of oxidative stress, induces high-anxiety-related behaviour in mice. Behavioural brain research, 359:292-297.

19. Zhang, C., Liu, Y., He, L., Shi, F., Yao, W., and Luo, X. (2021). Effects of uranium on the antioxidant responses of Chinese Oak Silkworm, Antheraea pernyi. Pakistan Journal Of Zoology. https://dx.doi.org/10.17582/journal.pjz/20190715000752

20. Lin, Y., Miao, L. H., Pan, W. J., Huang, X., Dengu, J. M., Zhang, W. X., and Xi, B. W. (2018). Effect of nitrite exposure on the antioxidant enzymes and glutathione system in the liver of bighead carp, Aristichthys nobilis. Fish & shellfish immunology, 76: 126-132.

21. Ayed, H. S., Khalaf, A. S., and Mohammed, T. M. T. (2021). Effect of Aqueous Extract of Truffle on Some Antioxidants in Rats Exposed to Oxidative Stress induced by Hydrogen peroxide. In IOP Conference Series: Earth and Environmental Science (Vol. 735, No. 1, p. 012019). IOP Publishing.

22. Ibuki, F. K., Bergamaschi, C. T., da Silva Pedrosa, M., & Nogueira, F. N. (2020). Effect of vitamin C and E on oxidative stress and antioxidant system in the salivary glands of STZ-induced diabetic rats. Archives of Oral Biology, 116: 104765.

23. Ma, L., Liu, J., Liu, A., and Wang, Y. (2022). Cytoprotective effect of Selenium polysaccharide from Pleurotus ostreatus against H2O2-induced oxidative stress and apoptosis in PC12 cells. Arabian Journal of Chemistry, 15(4): 1-12.

24. Al-Okialy, B. N. (2018). Role of Alpha Lipoic Acid in Oxidant/Antioxidant Status and Gene Expression of Glutathione Reductase in Hydrogen Peroxide Exposed Rats:(Part-2): 1Mohammad Saleh Alwan and 2Baraa Najim Al-Okialy. The Iraqi Journal of Veterinary Medicine, 42(2): 50-57

25. Chen, Z., Xing, T., Li, J., Zhang, L., Jiang, Y., and Gao, F. (2022). Oxidative stress induced by hydrogen peroxide promotes glycolysis by activating CaMKK/LKB1/AMPK pathway in broiler breast muscle. Poultry Science, 101(3), 101681.

26. Pedre, B., Barayeu, U., Ezeriņa, D., and Dick, T. P. (2021). The mechanism of action of N-acetylcysteine (NAC): The emerging role of H2S and sulfane sulfur species. Pharmacology & therapeutics, 228, 107916.

27. Singh, J., Phogat, A., Prakash, C., Chhikara, S. K., Singh, S., Malik, V., and Kumar, V. (2022). N-Acetylcysteine Reverses Monocrotophos Exposure-Induced Hepatic Oxidative Damage via Mitigating Apoptosis, Inflammation and Structural Changes in Rats. Antioxidants, 11(1): 90, https://doi.org/10.3390/antiox11010090

28. Chiu, C. H., Chang, C. C., Lin, S. T., Chyau, C. C., and Peng, R. Y. (2016). Improved hepatoprotective effect of liposome-encapsulated astaxanthin in lipopolysaccharide-induced acute hepatotoxicity. International journal of molecular sciences, 17(7): 1128, https://doi.org/10.3390/ijms17071128

29. Singh, A., Kukreti, R., Saso, L., and Kukreti, S. (2022). Mechanistic Insight into Oxidative Stress-Triggered Signaling Pathways and Type 2 Diabetes. Molecules, 27(3): 950, https://doi.org/10.3390/molecules27030950

30. Ibrahem, R. T. I., Mahmmod, A. A. M., and Taqa, G. A. T. (2020). The protective and Antioxidant Effect of Catechin and Apigenin on Some Biochemical Parameters in Blood Serum of Rats Exposed to Oxidative Stress Induced by H2O2. College Of Basic Education Researches Journal, 16(3): 813-852.

31. Obaid, Q. A., AL-Ani, K. A. A., and Kadhum, R. J. (2021). Effect of Anti-oxidant Activity of Kiwi Fruit Against Oxidative Stress Induced by Hydrogen Peroxide in Male Mice. International Journal of Drug Delivery Technology, 11(4):1412-1416.

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

33. Sekhar, R. V. (2022). Gly NAC (Glycine and N-Acetylcysteine) Supplementation Improves Impaired Mitochondrial Fuel Oxidation and Lowers Insulin Resistance in Patients with Type 2 Diabetes: Results of a Pilot Study. Antioxidants, 11(1): 154, https://doi.org/10.3390/antiox11010154.

34. Keshk, W. A., Ibrahim, M. A., Shalaby, S. M., Zalat, Z. A., & Elseady, W. S. (2020). Redox status, inflammation, necroptosis and inflammasome as indispensable contributors to high fat diet (HFD)-induced neurodegeneration; Effect of N-acetylcysteine (NAC). Archives of biochemistry and biophysics, 680, 108227.

35. Zalewska, A., Ziembicka, D., Żendzian-Piotrowska, M., & Maciejczyk, M. (2019). The impact of high-fat diet on mitochondrial function, free radical production, and nitrosative stress in the salivary glands of Wistar rats. Oxidative Medicine and Cellular Longevity, Volume 2019, Article ID 2606120, 15 pages https://doi.org/10.1155/2019/2606120

36. Al-khafaf, A., Ismail, H. K., and Alsaidya, A. (2021). Histopathological effects of experimental exposure to lead on nervous system in albino female rats. Iraqi Journal of Veterinary Sciences, 35(1): 45-48.

37. Ahmed Aljanabi, M. S. A., Alfahdawi, O. A. S., and Ismail, T. F. (2018). Physiological and histological study of the effect of Cumin Cyminum watery extract and vitamin E on the male reproductive system in rats exposed to oxidative stress. Tikrit Journal of Pure Science, 23(3): 23-32.

38. Shamel, M., and Ankily, M. (2021). Histological and Ultrastructural Study of Silver Nanoparticles Toxicity and the Possible Protective Effect of Vitamin C on Submandibular Salivary Glands of Albino Rats. International Journal of Dentistry and Oral Sciencei.08(04):2166-2171.

39. Pei, Y., Liu, H., Yang, Y., Yang, Y., Jiao, Y., Tay, F. R. and Chen, J. (2018). Biological activities and potential oral applications of N-acetylcysteine: progress and prospects. Oxidative medicine and cellular longevity. https://doi.org/10.1155/2018/2835787

40. Tenório, M. C. D. S., Graciliano, N. G., Moura, F. A., Oliveira, A. C. M. D., & Goulart, M. O. F. (2021). N-Acetylcysteine (NAC): Impacts on Human Health. Antioxidants, 10(6): 967, https://doi.org/10.3390/antiox10060967

الملخص العربى

التأثير الوقائي لل ان اسيتيل سيستين على الغدد اللعابية ضد فرط الإجهاد التأكسدي في ذكور الجرذان البيضاء: دراسة كيميائية حيوية ونسيجية

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الهدف من الدراسة: دراسة الدور الوقائي المضاد للأكسدة لـ لل (ان اسيتيل سيستين NAC) ضد الإجهاد التأكسدي الناجم عن H_2O_2 في ذكور الجرذان البيضاء.

المواد والطرق: تم تقسيم أربعين من ذكور الجرذان البيضاء البالغة عشوائياً إلى ٤ مجموعات: المجموعة الأولى (مجموعة السيطرة السالبة) (ن = ١٠): نظام غذائي عادي ومياه الصنبور للشرب، وحقنت يومياً بـ ١ مل/كغم من الماء المقطر ((DW(بالبريتون) لمدة ٤ أسابيع. المجموعة الثانية: (المجموعة المعاملة بـ (H_2O_2 (ن = ١٠) تلقت نظامًا غذائيًا عاديًا، ومياه شرب تحتوي على ٠,٥٪ H_2O_2 يوميًا لتحفيز الإجهاد التأكسدي وتم حقنها يوميًا بـ ١ مل/كغم من وزن الجسم ماء مقطر لمدة ٤ أسابيع. المجموعة ٣: (مجموعة (NAC (ن = ١٠) تلقت نظامًا غذائيًا عاديًا ومياه الصنبور للشرب وتم حقنها يوميًا ب (NAC) ١٥٠ ملغم / كغم من وزن الجسم (بالبريتون) لمدة ٤ أسابيع. المجموعة ٤: (مجموعة الحماية) ((NAC+H٢O٢ (ن = ١٠) تلقت نظامًا غذائيًا عاديًا ومياه شرب تحتوي على ٠,٥٪ H٢٠٢ يوميًا للحث على الإجهاد التأكسدي وتم حقنها يوميًا بـ NAC ١٥٠ ملغم /كغم من وزن الجسم (بالبريتون) لمدة ٤ أسابيع. تم جمع عينات الدم من جميع مجاميع الحيوانات لاختبار إنزيمات مضادات الأكسدة (الكتاليز والجلوتاثيون بيروكسيديز) ومستوى السكر في الدم. تم فحص أوزان الجسم. وفي نهاية التجربة تم تخدير الحيوانات. تم التشريح للحصول على الغدد اللعابية للفحص النسيجي.

النتائج: أظهرت انخفاضاً ملحوظاً في مستويات كل من إنزيمي الكتاليز والجلوتاثيون بيروكسيديز وزيادة ملحوظة في مستوى الجلوكوز في الدم عند مستوى $p \leq 0.05$، وتدهور حاد في أنسجة الغدد اللعابية للمجموعة المعالجة بـ H_2O_2 مقارنة بمجموعة السيطرة السلبية، ومجموعه ال ان اسيتيل سيستين ومجموعة الحماية ($NAC+H_2O_2$) والتي لا تظهر أي تغييرات مهمة.

الاستنتاج: تشير هذه الدراسة إلى الدور الوقائي لـ NAC ضد تأثير الإجهاد التأكسدي الناجم عن H_2O_2 على أنسجة الغدد اللعابية والإنزيمات المضادة للأكسدة ومستوى السكر في الدم لدى ذكور الجرذان البيضاء.