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Research Article

Protective Effects of Vitamin U on Amiodarone-Induced Oxidative Stress and Altered Biomarkers in Rat Parotid Salivary Glands

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Abstract

Amiodarone (AMD), an antiarrhythmic drug, causes side effects in multiple organs. S-methylmethionine sulfonium (vitamin U, Vit U) has potential protective effects against these adverse outcomes. The objective of this study was to investigate the effects of AMD and Vit U on rat parotid salivary glands. The rats were assigned to four groups: control (corn oil for 7 days), Vit U (50 mg/kg/day for 7 days), AMD (100 mg/kg/day for 7 days), and AMD+Vit U (combined AMD and Vit U for 7 days). On day 8, parotid glands were collected for analysis of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), thromboplastic activitiy (TrA), lipid peroxidation (LPO), myeloperoxidase (MPO), sodium-potassium ATPase (Na^+/K^+-ATPase), sialic acid (SA), hexose, hexosamine, fucose and total protein (TP). AMD administration significantly increased the values of GSH, LPO, MPO and hexosamine while decreasing the values of Na^+/K^+-ATPase, SA and hexose compared to controls. Co-administration of Vit U with AMD reversed these changes, indicating that Vit U may help to reduce AMD-induced oxidative stress in the parotid salivary gland and restore altered biochemical parameters. 7-day treatment with AMD induced oxidative damage and inflammation in the parotid gland. Vit U showed protective effects, especially in reducing oxidative damage, inflammation and glycosylation changes.

Keywords: Amiodarone, inflammation marker, oxidative stress, parotid salivary gland, vitamin U

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Introduction

Amiodarone (AMD) is a commonly used class III antiarrhythmic medication for managing different cardiac rhythm disorders, including atrial fibrillation and ventricular tachycardia. Despite its high efficacy, AMD is known to cause a number of dose-dependent side effects affecting various organ systems, such as the lungs, liver, thyroid, and kidneys (Sarma et al 1997, Srinivasan et al 2019, You et al 2022, Fatima et al 2022, Şorodoc et al 2024). Furthermore, due to its strong lipid affinity, AMD tends to accumulate in tissues such as the liver, lungs, skin, eyes, adipose tissue, muscles (particularly the myocardium), and the thyroid gland, which accounts for its side effects on various organs (Narayana et al 2011). These side effects are often associated with oxidative stress, inflammation, and cellular damage (Bulut et al 2025). However, in contrast to this, Sarma et al (1997) found that oxidative stress is not involved in the pathogenesis of AMD toxicity. There is a debate in the literature about the oxidative potential of AMD. While some research show AMD's oxidant effects, others suggest AMD can inhibit lipid peroxidation (LPO), acting as an antioxidant molecule (Lapenna et al 2001). Among the potential mechanisms of AMD toxicity, oxidative stress has been identified as the primary factor underlying the drug's toxic effects (Moustafa et al 2020). Oxidative stress, specifically, is a key factor in the pathophysiology of AMD toxicity, as it leads to the production of reactive oxygen species

(ROS), which can harm lipids, proteins, and DNA (Abdelazim and Abomughaid 2024).

Glycoproteins, mainly referred to as fucose, hexose, and hexosamine, are crucial parts of cell membranes. They are made up of a protein moiety that is covalently attached to the side chain of a peptide. Glycoproteins mediate cellular adhesion, cell-cell recognition, and cell surface functions, many other functions. Many researchers have reported on the significance of glycoproteins and the elevated quantities of these proteins in a variety of disorders (Anandakumar et al 2008, Punithavathi and Prince 2009, Saravanan et al 2010). Oxidative damage caused by AMD has been found to disrupt cellular functions, impair antioxidant defense mechanisms, and contribute to inflammation and tissue damage (Şorodoc et al 2024). AMD has been also proposed as a hepatic mitochondrial uncoupling oxidative phosphorylation, to inhibit electron transport chain enzymes, and to hinder fatty acid β-oxidation (Lewis et al 1989, Waldhauser et al 2006, Felser et al 2013). Many organs' functions can be negatively impacted by disruptions to key physiological processes including mitochondrial β-oxidation and oxidative phosphorylation (Felser et al 2013). Consequently, there is increasing interest in identifying protective agents that can attenuate or prevent these deleterious effects.

Since oxidative stress plays an important role in the pathogenesis of AMD toxicity, antioxidants can be used to reduce these side effects. The antioxidant systems consist of both enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, and non-enzymatic antioxidants, such as glutathione (GSH), uric acid (Dunning et al 2013). These antioxidants play a key role in preventing tissue injury induced by oxidative stress (Halliwell 1991). In addition to directly damaging macromolecules like DNA, proteins, and lipids, excessive ROS production can alter several signaling pathways by inducing transcription factors that are sensitive to ROS. Antioxidant molecules are therefore required for these ROS to be detoxified (Wells et al 2009).

S-methylmethionine sulfonium, more commonly known as vitamin U (Vit U), is a naturally derived compund from the amino acid methionine. It is widely recognized for its potential antioxidant and anti-inflammatory properties that can counteract oxidative stress and protect against cellular damage (Bayrak et al 2022, Mahmarzayeva et al 2022, Topaloglu et al 2022). Vit U has historically been used for its hepatoprotective effects, particularly to treat gastric ulcers and support liver health (Abouzed et al 2021). Recent research suggests that Vit U may have broader protective effects by regulating oxidative stress and inflammation in other tissues, including the kidneys and lungs (Turkyilmaz and Yanardag 2016, Oztay et al 2020, Ak et al 2022). Preliminary studies suggest that Vit U may reduce LPO, restore antioxidant enzyme activity, and improve tissue integrity under conditions of oxidative stress (Gessler et al 1996). While the majority of researches have focused on its effects in the liver and gastrointestinal tissues, there is growing interest in investigating its potential to prevent drug-induced damage in other organs, including the salivary glands (Celik et al 2021, Topaloglu et al 2022). However, the potential protective effects of Vit U against damage induced by AMD in the parotid salivary gland, a critical organ involved in saliva production, have largely remained unexplored. In particular, the effects of AMD on the parotid salivary glands, which are essential for maintaining oral health through salivation, are poorly understood.

The parotid salivary gland is an exocrine gland that plays a vital role in the production of saliva, which is essential for digestion, lubrication, and protection of the oral cavity from pathogens, oral health, and immunity (Ungureanu et al 2023). Due to its significant metabolic activity, this gland is highly susceptible to oxidative damage, which can alter its biochemical composition and functional integrity (Shang et al 2023). Oxidative stress in the parotid gland can lead to secretory dysfunction, inflammation, and damage to cellular structures, potentially resulting in long-term dysfunction (Chibly et al 2022). Recent studies have shown that oxidative stress can impair salivary gland function by inducing LPO, altering enzyme activities, and changing the levels of key molecules involved in cellular signaling and protection, such as sialic acid (SA) and hexose (Zalewska et al 2020). Among the parameters used to assess oxidative damage in tissues, antioxidants are commonly measured and play crucial roles in protecting cells from oxidative damage by neutralizing ROS and preserving the balance of cellular redox (Cadenas and Davies 2000). Moreover, markers such as malondialdehyde (MDA) and myeloperoxidase (MPO) are frequently measured to evaluate the extent of oxidative damage and inflammation in tissues (Chen et al 2020, Cordiano et al 2023). Despite the increasing concern about AMD-induced damage to the salivary glands, limited studies have focused on its direct effects on the parotid salivary gland (Scully and Bagan 2004).

Considering the limited understanding of the effect of AMD on the parotid salivary glands and the potential protective effects of Vit U, this original study aimed to explore the biochemical changes induced by AMD in the parotid glands of rats and to evaluate whether Vit U

can attenuate these effects. By analyzing key markers, including GSH, SOD, CAT, thromboplastic activitiy (TrA), LPO, MPO, sodium-potassium ATPase (Na^+/K^+-ATPase), SA, hexose, hexosamine, fucose and total protein (TP), we aim to provide new insights into the protective role of Vit U against AMD-induced damage in the parotid salivary gland.

Material and Methods

Chemicals

The analytical-grade chemicals used in this research supplied by the following companies Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland). AMD (A8423) was purchased from Sigma-Aldrich and Vit U (64382) was from Fluka.

Devices

The devices used for biochemical analyses include a UV-Vis spectrophotometer (Rayleigh UV-1800), centrifuges (Nüve NF 200, Heraeus-Sepatech Labofuge 200, Heidolph), a homogenizator (Janke & Kunkel Ultra Turraxt 25), water baths (37°C, 100°C) (Boehringer – Mannheim, Nuve BT 400), a deep freeze (Soğuk Teknik, -80°C), an electronic balance (Shimadzu AUX 220), a distilled water and deionized water device (Purelab Option Q DV25), and a pH meter (Thermo Orion 710 A pH/ISE Meter).

Laboratory Animals and Experimental Design

Three and four months-old and weighing 200-220 g male Sprague-Dawley rats were used. The animals were housed in an animal room with optimum temperature (20°C ± 2), humidity, and 12 h light/12 h dark conditions. All rats were orally fed pellet-type rat food and fresh tap water. The dose and duration of AMD were determined based on the study by Reasor et al (1996). The Vit U dose was administered according to the protocol by Sokmen et al (2012). Male Sprague-Dawley rats were divided into four groups (n=6 per group): control group (corn oil given group), Vit U group (50 mg/kg/day Vit U for 7 days), AMD (100 mg/kg/day for 7 days), and AMD+Vit U (combined AMD and Vit U for 7 days). On day 8, rats were sacrificed under anesthesia, and parotid glands were collected.

Biochemical Examination

The homogenates were stored in a deep freezer at -80°C until analysis. The homogenates of salivary glands were centrifuged, and the supernatants were used for the analysis of all biochemical parameters except TrA. The homogenate was used directly for TrA analysis. Since clotting time is inversely proportional to TrA, a lengthening of the clotting time shows decreased TrA. The homogenates of parotid salivary glands were centrifuged, and the supernatants were used for the analysis of all biochemical parameters. GSH, SOD, CAT, LPO, MPO, Na^+/K^+-ATPase, SA, hexose, hexosamine, fucose and TP were determined in 10% (w/v) parotid salivary gland homogenates.

For the GSH determination, the absorbance of the colored product formed by the reaction of Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid) and sulfhydryl groups was spectrophotometrically recorded at 412 nm and calculated using the extinction coefficient (1.36×10^4 M^{-1} cm^{-1}) (Beutler 1975).

SOD activity was determined by riboflavin-sensitized photooxidation of *ortho*-dianisidine (Mylorie et al 1986). *Ortho*-dianisidine oxidation, by the reaction with riboflavin, is induced by SOD. The increase in absorbance depends on the SOD concentration. Finally, the colored product's absorbance was determined spectrophotometrically at 460 nm.

CAT activity was determined based on the H_2O formation reaction from H_2O_2. It was noted by the decrease in the absorbance obtained at 240 nm (Aebi 1984).

The absorbance of the pink color produced at the end of the reaction between the LPO product MDA and thiobarbituric acid was evaluated spectrophotometrically to determine LPO (Ledwozyw et al 1986).

MPO activity was measured using a method involving phenol, 4-aminoantipyrine, and H_2O_2. The absorbance was measured spectrophotometrically at 510 nm (Metcalf et al 1986).

The determination of Na^+/K^+-ATPase activity is based on measuring the inorganic phosphate released by the hydrolysis of ATP when the homogenate is incubated with an appropriate amount of ATP. For this, first total ATPase activity is determined. Then, in the absence of ouabain, Mg-ATPase is determined. The difference between them gives Na^+/K^+-ATPase activity (Ridderstap and Bonting 1969).

The levels of SA were evaluated using the method of Warren (1959). For the assay, salivary gland samples were first incubated at 80°C for 1 h with 0.1 N H_2SO_4, and the resulting hydrolysate was used for analysis. The absorbances obtained from the samples were determined at 549 nm.

The color produced by mucin (hexose, six-carbon carbohydrates) in the tissue homogenate with orcinol in a concentrated sulfuric acid medium was evaluated spectrophotometrically (Winzler 1955, Shakeerabanu et al 2011).

Hexosamines in the tissue homogenate are converted to pyrrole derivative by boiling with acetyl acetone. The color produced by the pyrrole derivative when reacted

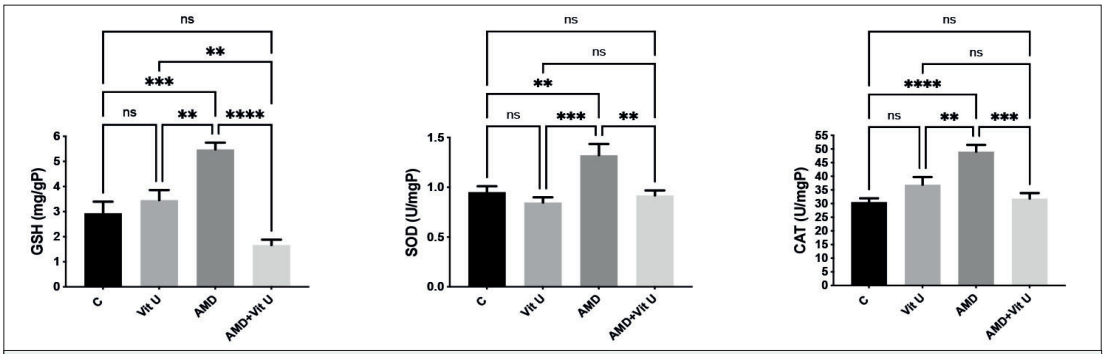

Figure 1. Parotid salivary gland GSH, SOD, and CAT values. Values were given as mean±SEM. SEM: Standard error of mean; C: Control; Vit U: Vitamin U; AMD: Amiodarone; P: Protein, GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase; **p<0.01, ***p<0.001, ****p<0.0001, ns: non-significant.

with ethyl alcohol and *p*-dimethylamino benzaldehyde (Ehrlich reagent) was evaluated spectrophotometrically (Winzler 1955, Shakeerabanu et al 2011).

The color produced by fucose (methylpentose) in the tissue homogenate with sulfhydryl compounds in a concentrated sulfuric acid medium was evaluated spectrophotometrically (Dische and Shettles 1948).

TrA in the salivary glands was evaluated according to the method of Ingram and Hills. In Quick's one-stage method, TrA in the homogenate was measured using pooled plasma obtained from healthy subjects. Since clotting time is inversely proportional to TrA, a lengthening of the clotting time shows decreased TrA (Ingram and Hills 1976).

TP levels in the samples were determined using the method of Lowry et al (1951), with bovine serum albumin as the protein standard. TP in parotid gland samples were assessed by using alkaline copper sulfate and phosphomolybdic-phosphotungstic acid reagent (Folin reagent) at 500 nm spectrophotometrically. The intensity of the blue color formed is proportional to the protein concentration. TP levels were used to express the results of the parameters per protein.

Statistical Analyses

Biochemical results were statistically evaluated via GraphPad Prism 9.0. The values were expressed as mean± standard error of the mean. Since there was a normal distribution, analysis of variance (ANOVA), an unpaired t-test, and Tukey's multiple comparison tests were used to assess the findings. Principal component analysis (PCA) was employed to illustrate the biochemical changes for all exposure conditions. The correlations between the biochemical parameters were also analyzed with Pearson correlation coefficient. The value of $p<0.05$ was considered statistically significant.

Results

GSH, SOD and CAT Values in Parotid Salivary Gland

Parotid salivary gland GSH, SOD and CAT values were seen in Figure 1. GSH, SOD and CAT values increased significantly in the AMD group compared to control ($p<0.001$, $p<0.01$, $p<0.0001$ respectively) and the Vit U group ($p<0.01$, $p<0.001$, $p<0.01$ respectively). GSH, SOD and CAT values decreased signifcantly in the AMD+Vit U groups compared to the AMD group ($p<0.0001$, $p<0.01$, $p<0.001$ respectively). The decrease in GSH values in the AMD+Vit U group was also significantly different from the Vit U group ($p<0.01$). No significant differences were found between the Vit U and AMD+Vit U groups in the SOD and CAT values ($p>0.05$).

TrA, LPO, MPO, Na^+/K^+-ATPase in Parotid Salivary Gland

Parotid salivary gland TrA, LPO, MPO and Na^+/K^+-ATPase values were seen in Figure 2. TrA were not significantly different between groups ($p>0.05$). LPO and MPO values increased significantly in the AMD group compared to the control (both $p<0.0001$), and Vit U groups (both $p<0.0001$). In the AMD+Vit U group, LPO and MPO values decreased significantly compared to the AMD group (both $p<0.0001$). Na^+/K^+-ATPase values in the Vit U, AMD and AMD+Vit U groups decreased significantly compared to the control group ($p<0.0001$, $p<0.0001$, $p<0.05$ respectively).

SA, Hexose, Hexosamine and Fucose Values in Parotid Salivary Gland

The SA, hexose, hexosamine, and fucose values were seen in Figure 3. SA and hexose values in the AMD group decreased significantly compared to the control group ($p<0.01$ and $p<0.001$, respectively). AMD+Vit U treatment caused a significant increase in SA values compared to the

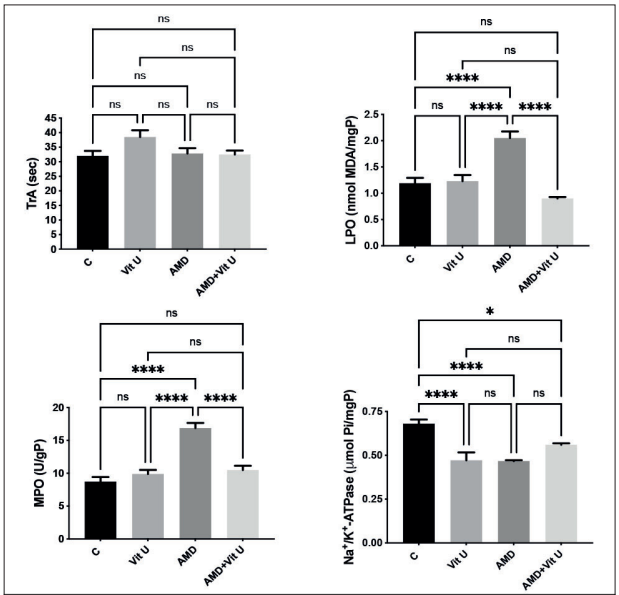

Figure 2. Parotid salivary gland TrA, LPO, MPO, Na^+/K^+-ATPase values. Values were given as mean±SEM. SEM: Standard error of mean; C: Control; Vit U: Vitamin U; AMD: Amiodarone; P: Protein, Sec: Second, Pi: Inorganic phosphate, LPO: Lipid peroxidation; TrA: Tromboplastic activity; MPO: Myeloperoxidase; Na^+/K^+-ATPase: Sodium-potassium ATPase; *p<0.05, ****p<0.0001, ns: non-significant.

AMD group (p<0.001). However, hexose values decreased significantly with AMD+Vit U treatment (p<0.05). There were significant increases in the hexosamine values in both the AMD and Vit U groups compared to the control group (p<0.0001 and p<0.05, respectively). AMD+Vit U treatment caused significant decreases in hexosamine values compared to the AMD and Vit U groups (p<0.0001 and p<0.01, respectively). Furthermore, AMD+Vit U treatment resulted in significant decreases in fucose values compared to the control and AMD groups (p<0.001and p<0.01, respectively).

Principal Component Analysis (PCA)

The PCA was performed on all groups to explain the effects of Vit U and AMD on the biochemical parameters measured in the parotid salivary gland (Figure 4A and 4B). The correlation coefficients between parameters are seen in Figure 4C. PCA revealed that the first two components detailed around 68.98% of the total variation in the experimental data. PC1 and PC2 explained 49.91% and 19.07% of the total variance, respectively. According to the PCA results, MPO, CAT, hexosamine, SOD, GSH, LPO, fucose and TrA were clustered together and negatively correlated with SA, Na^+/K^+-ATPase and hexose.

Discussion

This experimental study investigates the effects of AMD and Vit U on oxidative stress, antioxidant enzyme activities, LPO, glycosylation markers and cellular function in the rat parotid salivary gland. The parotid gland, a major salivary gland, plays an important role in maintaining oral health and general well-being, and its dysfunction may be associated with oxidative stress and inflammation (Zalewska et al 2020, Chibly et al 2022, Ungureanu et al 2023). The findings of this study provide valuable insight into the effects of AMD and Vit U, both individually and in combination, on the parotid gland, with particular focus on oxidative damage and the potential protective role of Vit U.

This study used male Sprague-Dawley rats, which are widely used in pharmacological and toxicological studies due to their well-characterized physiology and responsiveness to experimental treatments (Foster and Frost 2015, Clements et al 2022). The AMD dose used in this study, 100 mg/kg/day, is consistent with doses used in other animal studies examining AMD-induced toxicity and oxidative stress (Oktay et al 2018, Hazineci et al 2020, Turkyilmaz 2023). This dose is sufficient to induce oxidative stress and inflammation without causing acute toxicity and allows researchers to assess antioxidant defense mechanisms in response to AMD exposure. In the study by Al-Shammari (2016), Sprague-Dawley rats have been treated with AMD doses of 80 mg/kg/day intraperitoneally for ranging from one to four weeks. A dose of 50 mg/kg/day of Vit U has also been used in previous studies examining its antioxidant properties (Sokmen et al 2012, Baş et al 2016, Oktay et al 2018, Hazineci et al 2020, Turkyilmaz 2023) and is considered

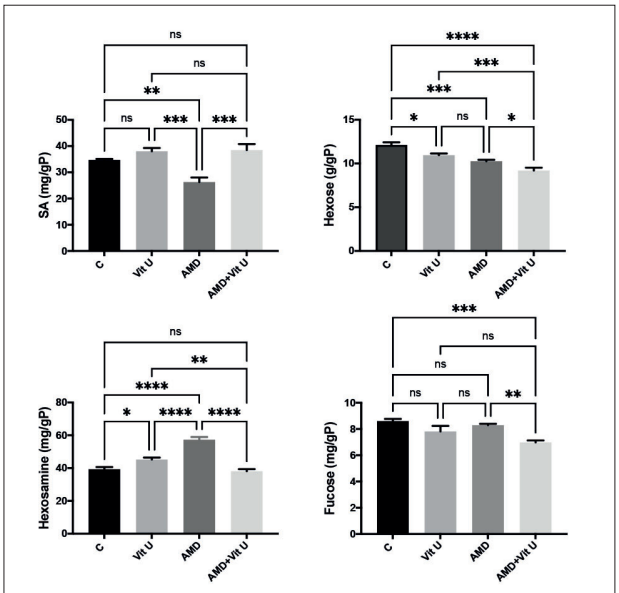

Figure 3. Parotid salivary gland SA, hexose, hexosamine and fucose values. Values were given as mean±SEM. SEM: Standard error of mean; C: Control; Vit U: Vitamin U; AMD: Amiodarone; P: Protein, SA: Sialic acid; *p<0,05, **p<0.01, ***p<0.001, ****p<0.0001, ns: non-significant.

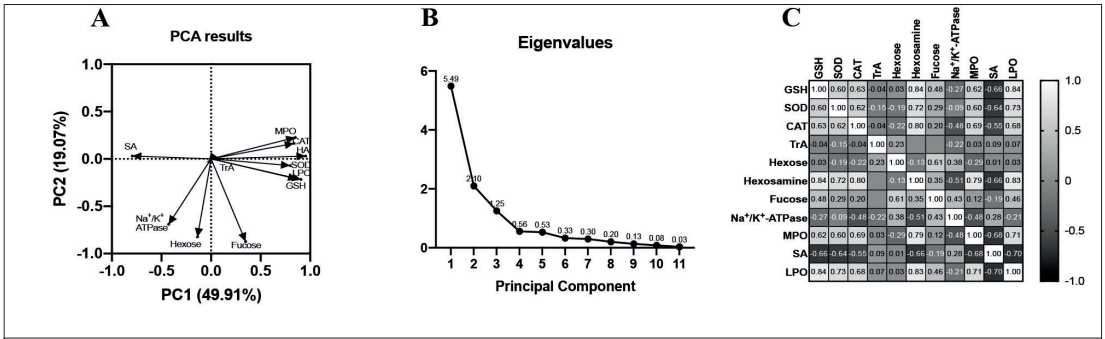

Figure 4. Principal component analysis (PCA). PCA results (A), eigenvalues (B) and correlation matrix (C) of biochemical parameters of parotid gland. PC1: 49.91% and PC2: 19.07%, Total PCA result: 68.98%. GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase; LPO: Lipid peroxidation; TrA: Tromboplastic activity; MPO: Myeloperoxidase; Na^+/K^+-ATPase: Sodium-potassium ATPase; SA: Sialic acid; HA: Hexosamine.

effective dose to attenuate oxidative damage. A 7-day treatment period was sufficient to observe acute changes in oxidative stress and inflammation markers but longer treatment periods may provide additional information on the chronic effects of AMD and the long-term protective effects of Vit U.

Oxidative stress typically arises from an overproduction of ROS or a reduction in the cell's antioxidant defense mechanisms. AMD administration results in a decline in GSH, SOD, and CAT levels, either individually or collectively, in various organs, including the heart (Hazineci et al., 2020), brain (Turkyilmaz 2023), liver (Baş et al 2016), and lungs (Moustafa et al 2020). However, in contrast to these studies, the present study found significant increases in parotid gland GSH, SOD, and CAT levels in the AMD group compared to the control and Vit U groups. As the parotid gland presents different metabolic profiles at rest and under stimulation from the submandibular gland (Nogueira and Carvalho 2017, Carvalho et al 2019), and has a more efficient antioxidant system (Nogueira et al 2005, Ibuki et al 2010), it may display characteristics that differ from those of other tissues as well. In the present study, it was thought that AMD may induce oxidative stress in the parotid gland and this may lead to an adaptive increase in antioxidant defense systems. Golli-Bennour et al (2012) revealed that AMD can lead to an increased in ROS production and subsequently activate antioxidants due to its pro-oxidant properties. In the study conducted by Ak et al (2022), increased activities of SOD, CAT, glutathione peroxidase and glutathione reductase have been found in AMD treated lung tissues. This elevation has been thought as an indicator for the deleterious effects on mitochondrial process of AMD. Similarly, another study found that oxidative stress-induced tissue damage resulted in a notable rise in SOD and CAT activity as well as MDA and TOS levels, whereas GSH and TAS levels declined (Cosgun et al 2019) showing compatibility with Gangarapu et al (2013), El-Meligy et al (2014), Ozturk et al (2003), Sarıpınar Aksu et al (2016) and Ranjbar et al (2014) studies. In the present study, the increased GSH, SOD, and CAT values observed in the AMD group are consistent with these findings and suggest that the parotid gland attempts to counteract the oxidative stress induced by AMD.

Antioxidants like Vit U may regulate antioxidant enzymes to prevent excessive cellular responses (Turkyilmaz and Yanardag 2019, Oztay et al 2020, Fatima et al 2022, Bayrak et al 2022, Turkyilmaz 2023). Our data also showed that the combination of AMD+Vit U caused a significant decrease in GSH levels compared to the AMD alone group. This suggests that Vit U may modulate the oxidative stress response by altering cellular redox signaling pathways or by preventing excessive activation of the antioxidant system. Despite the antioxidant properties of Vit U, the decrease in GSH levels in the AMD+Vit U group suggests a complex interaction between AMD-induced oxidative stress and the protective effects of Vit U. It is possible that Vit U works to restore redox balance and prevent an overcompensatory response, rather than further increasing antioxidant enzyme levels.

LPO and MPO are both markers of oxidative damage and inflammation (Wilkie-Grantham et al 2015, Chen et al 2020, Cordiano et al 2023) and were significantly increased in the AMD group compared to the C and Vit U groups. High LPO levels in the AMD group indicate extensive damage to cell membranes, which is a marker of oxidative stress (Turkyilmaz and Yanardag 2019). MPO, an enzyme involved in the production of ROS during inflammation (Turkyilmaz and Yanardag 2019), was also significantly increased in the AMD group, suggesting that AMD induces an inflammatory response in addition to oxidative damage. These findings are consistent with previous studies showing that AMD induces both oxidative stress and inflammation in various tissues

(Moustafa et al 2020, Dawood et al 2024). Interestingly, the combination treatment with AMD and Vit U resulted in a significant decrease in both LPO and MPO values compared to the AMD alone group. This suggests that Vit U has a protective effect against oxidative damage and inflammation in the parotid gland. The antioxidant and anti-inflammatory properties of Vit U, as evidenced by the decrease in both LPO and MPO, are consistent with its known ability to attenuate ROS-induced damage and inflammatory responses (Oktay et al 2018, Hazineci et al 2020, Turkyilmaz 2023). These findings underline the therapeutic potential of Vit U in reducing oxidative damage and inflammation induced by pharmacological agents like AMD.

AMD-induced oxidative stress leads to ion pump dysfunction in various tissues (Gray et al 1998, Pitt et al 2003). In this study, Na^+/K^+-ATPase activity was significantly decreased in all treated groups AMD, Vit U and AMD+Vit U compared to the control group. Na^+/K^+-ATPase plays an important role in maintaining cellular ion homeostasis, and its dysfunction is a common consequence of oxidative stress (Clausen et al 2017, Lichtstein et al 2018). The decreased Na^+/K^+-ATPase activity in the AMD group is likely due to oxidative damage caused by increased ROS production. In a study by Turkyilmaz (2023), Vit U administration increased Na^+/K^+-ATPase activity in AMD given Vit U group as compared to the AMD group. It has been suggested that this effect may be due to membrane repair and antioxidant effects of Vit U, as earlier indicated by Rácz et al (2008), Turkyilmaz and Yanardag (2016) and Topaloglu et al (2022). In the present study, the lack of improvement in Na^+/K^+-ATPase activity in the AMD+Vit U group suggests that although Vit U is effective in reducing oxidative damage and inflammation, it may not fully restore ion transport mechanisms in the parotid gland. This may indicate that oxidative stress-induced damage to Na^+/K^+-ATPase requires longer periods or higher doses of Vit U for full restoration. Alternatively, the impairment of Na^+/K^+-ATPase may be independent of oxidative stress and may involve other cellular mechanisms (Silva et al 2021).

SA and hexoses play important roles in maintaining cell structure and function. SA is a terminal sugar residue found on glycoproteins and glycolipids that affects cell-cell interactions, immune responses, and cell signaling. Changes in SA levels may reflect changes in glycosylation patterns, which are often disrupted under conditions of oxidative stress or inflammation (Varki 2008). Hexoses, including glucose, fructose, and hexosamines, are key components of glycosaminoglycans and proteoglycans, which are important extracellular matrix components that support tissue integrity (Zhang et al 2022, Xu et al 2024). Hexosamines, in particular, play a role in cellular signaling pathways and the regulation of inflammation and stress responses (Paneque et al 2023, Zou et al 2023).

Significant changes in glycosylation markers including SA, hexose, hexosamine, and fucose, in the AMD groups suggest that AMD interferes with normal glycosylation processes in the parotid gland. The decrease in SA levels in the AMD group may reflect oxidative damage to glycosylation pathways. Similarly, changes in hexose and hexosamine levels suggest that AMD affects glycosylation of proteins, which may impair cellular function and signaling. The increase in SA levels in the AMD+Vit U group compared to the AMD group suggests that Vit U may help restore some aspects of glycosylation impaired by AMD. The antioxidant properties of Vit U may protect glycosylation pathways from oxidative damage and potentially improve cell surface function and signaling (Turkyilmaz and Yanardag 2016). However, the decrease in hexose values in the AMD+Vit U group suggests that Vit U may selectively modulate specific glycosylation pathways, possibly to maintain cellular homeostasis. The observed changes in fucose and hexosamine levels also support the idea that Vit U has the potential to regulate glycosylation processes and provide a novel mechanism for its protective effects.

TrA is a low molecular weight glycoprotein that is a part of the cell membrane and is also known as tissue factor (TF). It is the principal cellular initiator of normal blood coagulation. Higher blood coagulation levels and a shorter coagulation time are correlated with an increase in TrA. Changes in the cell membrane, either as a result of elevated LPO levels or modifications in the membrane's composition, are also linked to this enhanced activity (Bächli 2000, Danese et al 2007). A number of diseases, including diabetes, hyperlipidemia, atherosclerosis, and kidney disorders, have been linked to elevated TrA (Minuz et al 2002, Emekli-Alturfan et al 2007). According to Breitenstein et al.'s study of AMD's effects on thrombus formation and TF expression, AMD prevents thrombus formation in vivo (Breitenstein et al 2008). In the study by Turkyilmaz and Yanardag (2016), TrA has been found to increase significantly in the AMD given group when compared to the control group, and Vit U reversed this activity in AMD group. They have suggested that this reduction in TrA can be associated with the protective effect of Vit U on membrane stability (Rácz et al 2008). On the contrary, in the present study, TrA were not significantly different between the groups.

PCA performed on experimental data provides valuable insights into the biochemical changes induced by AMD and Vit U treatments in the parotid salivary gland. PCA is a powerful statistical tool used to reduce the dimensionality of complex data sets and reveal patterns in data by transforming correlated variables into a smaller

set of uncorrelated components (Greenacre et al 2022). In this study, the first two components, PC1 and PC2, explain approximately 69% of the total variation in the data, highlighting their important role in understanding the underlying patterns in biochemical markers. The clustering of MPO, CAT, hexosamine, SOD, GSH, LPO, fucose, and TrA suggests that these parameters are affected by similar treatment effects and are associated with oxidative stress and metabolic changes. These findings emphasize the role of oxidative stress markers in the overall biochemical response to treatments, which is consistent with the observed changes in GSH, SOD, and CAT levels in different groups. Furthermore, negative correlations with SA, Na^+/K^+-ATPase, and hexose provide insight into the broader metabolic changes induced by AMD and Vit U, indicating a potential shift in cellular functions such as ion transport and glycosylation.

In terms of interpretation, the PCA results highlight the complex and multifaceted effects of AMD and Vit U. The significant clustering of oxidative stress-related parameters, such as MPO, LPO and SOD, suggests that oxidative damage plays a central role in the observed changes, particularly in group AMD. It is noteworthy that the AMD+Vit U treatment led to a decrease in oxidative stress markers compared to group AMD, which may indicate a protective effect of Vit U. However, the decreases in SA and fucose levels in the AMD+Vit U group may indicate dysregulation of glycosylation pathways that may be affected by these treatments. These findings provide a comprehensive view of the biochemical landscape affected by AMD and Vit U, opening avenues for further investigation of the molecular mechanisms driving changes in oxidative stress, glycosylation and ion transport. It will be interesting to investigate the functional implications of these changes, particularly in the context of salivary gland function and broader systemic effects.

Limitations of the present study are the lack of the analysis of more inflammatory markers and the blood biochemical parameters. Future research should incorporate histopathological examinations to more clearly define the effects of AMD and Vit U at the tissue level in the parotid salivary gland.

Conclusion

In conclusion, the results of this experimental study provide valuable information on the effects of AMD and Vit U on oxidative stress, antioxidant enzyme activity, LPO, glycosylation markers, and cellular function in the rat parotid salivary gland. 7-day treatment with AMD (100 mg/kg/day) and Vit U (50 mg/kg/day) induced oxidative stress and inflammatory responses in the parotid gland. Vit U showed protective effects, especially in reducing oxidative damage, inflammation and glycosylation changes. However, Vit U did not fully restore all aspects of cellular function, such as Na^+/K^+-ATPase activity. These findings highlight the need for further studies to investigate the long-term effects of Vit U and its potential to reduce AMD-induced toxicity in other tissues.

Declarations

Competing Interests

Authors declare that there are no conflicts of interest related to the publication of this article.

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A preliminary analysis of some of the data reported in this work was presented as a poster presentation at the 2nd National Glycobiology Congress, on June 11-14, 2013.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

All experiments in this study were approved by the Marmara University Experimental Animals Ethics Committee (Decision No: 135.2013.mar).

Author Contributions

Motivation / Concept: AY, RY; Design: AY, RY, SA; Control/ Supervision: AY, RY; Data Collection and Processing: BAT, IBTM, SO, SK; Analysis and Interpretation: AY, RY, BAT, IBTM; Literature Review: BAT, IBTM, AY, RY; Writing the Article: AY, BAT, IBTM; Critical Review: AY, RY, BAT, IBTM, SO, SK, SA

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