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Are Antibiotics Sufficient for Treating Bacterial Rhinosinusitis? The Influence of Alpha-Lipoic Acid, a Potent Antioxidant, As an Additional Treatment in Bacterial Rhinosinusitis

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ABSTRACT

Objective: Alpha-lipoic acid is an antioxidant agent with potential anti-inflammatory properties and is produced from octanoic acid. The aim of this study was to investigate the effects of alpha-lipoic acid on inflammation, oxidative status, tissue integrity in an animal model of experimentally induced acute rhinosinusitis and to compare these effects with the standard treatment, cefalosporin.

Methods: Totally 30 healthy Wistar Albino rats were used in the experiment. The animals were randomly divided into 6 groups. An experimental sinusitis model was created Staphylococcus Aureus (SA) in the groups except for the healthy group. Over a 10-day period, groups were treated daily either with 50 mg/kg cefazolin (SA + cefazolin), alpha-lipoic acid 100 mg/kg (SA + alpha-lipoic acid 100), alpha-lipoic acid 200 mg/kg (SA + alpha-lipoic acid 200), or alpha-lipoic acid 200 mg/kg + cefazolin 50 mg/kg (SA + alpha-lipoic acid 200 + cefazolin). At the end of the test, the animals were euthanized, and the maxillary sinus mucosa was removed. Mucosa samples were examined for superoxide dismutase activity and glutathione, malondialdehyde, tumor necrosis factor-alpha mRNA, and interleukin-1β mRNA levels.

Results: Histopathological examination showed lesser changes in SA + cefazolin group compared to the control group and inflammation proportionally with alpha-lipoic acid dose in rhinosinusitis-induced groups treated with alpha-lipoic acid. Increased levels of malondialdehyde, tumor necrosis factor-alpha mRNA, and interleukin-1β mRNA in rhinosinusitis-induced groups approached the healthy group in SA + alpha-lipoic acid 200 + cefazolin group. Decreased superoxide dismutase activity and glutathione level in induced rhinosinusitis groups were close to that of healthy group in SA + alpha-lipoic acid 200 + cefazolin group.

Conclusd'n: The results of the study revealed that using a potent antioxidant and/or anti-inflammatory agent along with an antibacterial agent could be more effective in reducing oxidative stress and cytokine levels in the treatment of bacterial infections like sinusitis.

Keywords: Alpha-lipoic acid, antibiotics, antioxidant, bacterial rhinosinusitis

Introductd'n

Acute rhinosinusitis is a quite common disease characterized by the inflammation of nasal and para-nasal sinus mucosa.[1] *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Moraxella catarrhalis* are the most common etiologic agents in acute rhinosinusitis.[2] The natural immune response is activated after the body recognizes the bacterial pathogen. Acute inflammatory response begins with the release of various cytokines (tumor necrosis factor-alpha [TNF-α]), interleukins

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([IL-1]), and chemokines IL-8, monocyte chemo-attractant protein-1).[3,4] These mediators rapidly induce the aggregation of inflammatory cells at the infection site.[3] Degranulation of the activated inflammatory cells leads to a rapid release of reactive oxygen species (ROS) like superoxide anion radical and hydrogen peroxide radical.

A physiologic balance is present between the production and elimination of (redox homeostasis) ROS in most of the cellular processes of the organism.[3-5] This balance is preserved with endogenous antioxidant mechanisms including superoxide dismutase (SOD), glutathione peroxidase, catalase, glutathione (GSH,), and peroxiredoxins.[6] Homeostasis is impaired in case of ROS over-expression resulting in oxidative stress. Oxidative stress causes a series of chemical reactions that play an important role in the pathophysiology of inflammation. Many studies have shown that oxidative stress can lead to the continuation and progression of chronic inflammation.[3-5] Some excessive oxidative stress conditions including sinusitis may lead to cell death and the damaging of extracellular membrane and finally tissue damage.[7,8] Malondialdehyde (MDA), a lipid peroxidation product, is a good marker of cellular damage.[9]

Various animal models of acute sinusitis have revealed that pharmacologic agents like *Nigella sativa* and curcumin which have anti-inflammatory and antioxidant properties could be a promising option for acute sinusitis treatment.[10,11] In a rat study of experimentally induced acute sinusitis, it was reported that epithelial tissue was at nearly normal appearance and cellular inflammation decreased in connective tissue in the group treated with curcumin.[10] In another study, *N. sativa* was reported to be able to prevent the histopathological changes of rhinosinusitis dose-dependently.[11] Based on these studies, it seems that novel studies are required to show the effectiveness of different pharmacologic agents with potent anti-inflammatory and antioxidant properties in acute sinusitis treatment. Oxidative stress, which develops after bacterial damage, impairs tissue integrity, as well as clinical and histopathological findings could continue although bacterial colonization could be eliminated with antibiotic treatment.[12]

Alpha-lipoic acid is an antioxidant agent with potential anti-inflammatory properties from octanoic acid in mitochondria and does not have severe potential side effects.[13] Alpha-lipoic acid is a cofactor of mitochondrial dehydrogenase complex and a physiologic compound of mitochondrial membranes.[14,15] It is active in both lipid and watery environments, moves both in extra- and intracellular environments, and thereby has a wide potential pharmacological effect.[16,17] Alpha-lipoic acid neutralizes free oxygen species like superoxide radicals, hydroxyl, and peroxyl radicals.[17,18] Alpha-lipoic acid was shown to be able to inhibit pro-inflammatory cytokines that were induced by lipopolysaccharides.[19] The benefits of ALA have been shown in many diseases like encephalomyelitis, optic neuritis, organ transplantation, diabetic neuropathy, Wilson's disease, ulcer, sepsis, and osteoporosis due to its anti-inflammatory and antioxidant properties.[14,20-26]

The increased antibiotic resistance and resultant decreased effectiveness of antibiotics made the investigation of non-antibiotic compounds like ALA with antioxidant and potential anti-inflammatory properties necessary. We believe that the antioxidant effects of ALA could be beneficial in rhinosinusitis treatment. The aim of this study was to investigate the effects of ALA on inflammation, oxidative status, tissue integrity in an animal model of experimentally induced acute rhinosinusitis and compare these effects with one of the most preferred treatment options, cefalosporin. In addition, we aimed to evaluate the potentializing effect of ALA on cefalosporin during rhinosinusitis.

Main Points

- Various animal models of acute sinusitis have revealed that pharmacologic agents which have anti-inflammatory and antioxidant properties could be an option for acute sinusitis treatment.
- Alpha-lipoic acid (ALA) is an antioxidant agent with potential anti-inflammatory properties.
- Our study showed that in the treatment of bacterial sinusitis, co-administration of ALA with an antibacterial agent can be effective in reducing oxidative stress and cytokine levels.

Methods

Animals

Totally 30 healthy Wistar Albino rats were used in the experiment. All animals were obtained from the Animal Laboratory of Atatürk University Medical Experimental Research and Training Center. The animals weighed 200-300 g. Animal tests and procedures were performed in accordance with the national guidelines concerning the usage and care of laboratory animals. The study was approved by Atatürk University Animal Tests Local Ethics Committee (project number: 2019-4/55). The Wistar Albino rats were placed in an air-conditioned room in standard plastic cages on sawdust beds at 22°C ± 1°C, 12 : 12 hour dark and light cycle. Animals were given standard animal food and provided with water. The duration of adaptation was 2 weeks prior to the test. The animals were randomly divided into 6 groups with 5 animals in each. In 1 group (the healthy group), experimental rhinosinusitis was not induced with *S. pneumoniae* serotype 3; in 1 group, experimental rhinosinusitis was induced with *S. pneumoniae* serotype 3 and no treatment was applied (SA); in 1 group, experimental rhinosinusitis was induced with *S. pneumoniae* serotype 3 and treated with intraperitoneal (IP) cefazolin (CEFA) sodium 50 mg/kg daily for 10 days (SA + CEFA); in 1 group, experimental rhinosinusitis was induced with *S. pneumoniae* serotype 3 and treated with oral ALA 100 mg/kg daily for 10 days (SA + ALA 100); in 1 group, experimental rhinosinusitis was induced with *S. pneumoniae* serotype 3 and treated with oral ALA 200 mg/kg daily and IP CEFA sodium 50 mg/kg daily for 10 days (SA+ALA 200+CEFA). On the 10th day of treatment, the animals were euthanized with 50 mg/kg thiopental anesthesia.

Animal Model

Merocel (Medtronic Xomed, Jacksonville, Fla, USA) buffer was shaped with micro scissors in a proper size for the nasal cavity and cut. All rats except the healthy group were administered ketamine (intramuscular (IM), 50 mg/kg; Ketalar Pfizer, Istanbul, Turkey) and diazepam (IM, 2 mg/kg; Diazem, Deva, Istanbul, Turkey). The nasal dorsum of the rats was sterilized with povidone-iodine. Merocel rods were placed into the right nasal cavities of the animals manually by sliding in the nostril

with the aid of micro forceps in all groups except the healthy group. *S. pneumoniae* serotype 3 (No. 49619, obtained from the university) was suspended with sterile saline (900×10^6 colony-forming units for each mL). 0.5 mL of *S. pneumoniae* suspension was dropped onto Merocel rods using a hypodermic syringe, and the rods were removed from the nasal cavities 24 hours after the bacterial inoculation.[27,28] Purulent nasal secretions were observed in the nasal cavities of the animals on the third day. Therapeutic intervention started on the fifth day. Healthy group and the SA group received both oral and IP vehicles which do not contain CEFA or ALA. The SA+CEFA group was treated with IP CEFA sodium 50 mg/kg daily; the SA + ALA 100 group was treated with oral ALA 100 mg/kg daily; the SA + ALA 200 group was treated with oral ALA 200 mg/kg daily; and the SA + ALA 200 + CEFA group was treated with oral ALA 200 mg/kg daily and IP CEFA sodium 50 mg/kg daily for 10 days. Alpha-lipoic acid was given in doses (100 mg/kg and 200 mg/kg) with high bioavailability and effectiveness according to the literatüre.[29] On the 10th day of treatment, the animals were euthanized with thiopental anesthesia and decapitated, a nasal dorsum skin elevation was performed, and the right nasal lateral walls and the right maxillary sinuses were removed. Some of the mucosa samples were stored at –80°C for biochemical analysis.

Histological Examinatd'n

Rat nasal tissues were rapidly fixed in 10% buffered formalin for 24-48 hours for histologic examination. Each nasal sample was embedded in paraffin, sectioned at 5 µm thicknesses, and stained with hematoxylin and eosin (H&E). All nasal tissues were examined by 2 independent researchers for histological evaluation of the following parameters: H&E staining for inflammatory cell infiltrates, epithelial degeneration, necrotic cells, and mucosal edema. Nasal smears were prepared on the slide using a swab from the nasal mucosa and were stained with H&E. Histopathologic findings were determined and ranked as: absent (0), mild (1), moderate (2), or severe (3) (Table 1), in randomly 6 microscope fields. After examinations, all sections and smears were photographed by a light photomicroscope.

Bd'chemical Examinatd'n of Sinonasal Mucosal Tissues

The sino-mucosal tissue samples were stored at –80°C following macroscopic analysis. All tissue samples were ground with fluid nitrogen using a Tissue Lyser II Jar grinding set (Qiagen, Hilden, Germany). Approximately 20 mg of ground tissue was homogenized and mixed with 1 mL phosphate buffer saline (PBS) in an Eppendorf tube using TissueLyser II, and 4000 g was centrifuged for 20 minutes afterward. For each tissue sample, GSH levels,[30] MDA levels,[31] protein levels, and SOD activities[32] were measured consecutively with an enzyme-linked immunosorbent assay (ELISA) reader. A standard curve was plotted and the equation was acquired from the absorbance of standards. Glutathione, MDA, SOD, and protein proportions were computed appropriating to this equation. The mean values absorbance of the samples was computed. The GSH, SOD, and MDA levels were given as nmol/mg, and the protein level was given as U/mg. The obtained data were presented as a mean ± standard deviation (SD)/mg protein.

SOD Activity

Superoxide dismutase activity was measured in accordance with Sun et al.[32] The SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium to form formazan dye. Superoxide dismutase activity was then measured at 560 nm by the degree of inhibition of this reaction and is expressed as U/mg protein.

Table 1. Histopathological Scores in Line with Histopathological Findings

Histopatological Scores	Score Grade
Inflammatory cell infiltrates	(0) None (1) 1-3 inflammatory cells (2) 3-6 inflammatory cells (3) >6 inflammatry cells
Epithelial degeneration	(0) None (1) Lesion in 1 microscope field (2) Lesions in 2-3 microscope fields (3) Lesions in 4-5 microscope fields
Necrotic cell death	(0) None (1) 1-3 necrotic cells (2) 3-6 necrotic cells (3) >6 necrotic cells
Mucosal edema	(0) None (1) <5% in microscope field (2) 5%-20% in microscope field (3) >20% in microscope field

Total GSH Determinatd'n

The amount of GSH in the tissues was measured according to the method described by Sedlak and Lindsay.[30] The tissues were weighed and homogenized in PBS buffer. The homogenate was then centrifuged. After centrifugation at 4200 rpm for 40 minutes at 4°C, the supernatant was used to determine the GSH using 5,5′-dithiobis-(2-nitrobenzoic acid). Absorbance was measured at 412 nm using an ELISA reader. The results of the GSH level in tissues were expressed as nmol/mg protein.

Determinatd'n of MDA

Malondialdehyde in tissue was determined by estimating the level of MDA using the thiobarbituric acid test with Ohkawa et al.[31] The tissues were weighed and homogenized in PBS buffer. The homogenate was added to a solution containing 0.2 mL of 80 g/L sodium lauryl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL of distilled water. The mixture was incubated at 98°C for 1 hour. After the mixture had cooled, 5 mL of *n*-butanol : pyridine (15 : l) was added. The mixture was centrifuged for 30 minutes at 4000 rpm. The supernatant was measured at 532 nm, and a standard curve was obtained using 1,1,3,3-tetramethoxypropane. The results were expressed as nmol/mg protein.

Protein Detectd'n

Protein concentration was detected using commercial protein standards by the Lowry method (total protein kit-TP0300-1 KT; Siga Chemical Co, Munchen, Germany).

Real-Time PCR

Twenty milligrams of homogenized tissue were stabilized in RNA stabilization reagent (RNAlater; Qiagen) and then

disrupted using the TissueLyser II (Qiagen). The total RNA was purified using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions in a QIAcube (Qiagen). The RNA samples then were reverse-transcribed into complementary DNA with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, Calif, USA). The cDNA concentration and quality were assessed and quantified using the Epoch Spectrophotometer System and Take3 Plate (BioTek, Winooski, Vt, USA).

Relative Quantificatd'n of Gene Expressd'n

Analysis of relative TNF-α and IL-1 mRNA expression was performed using StepOnePlus Real-Time PCR System technology (Applied Biosystems) using cDNA synthesized from rat RNA. The following primers purchased as assay-on-demand from Applied Biosystems (Nieuwerkerk a/d IJssel, The Netherlands) were used for real-time PCR: rat TNF-α (Rn00562055_m1), rat IL-1β (Rn00580432_m1), and rat β-actin Rn00667869_m1. The real-time reverse transcriptase-polymerase chain reaction (qPCR) was run using the Primer Perfect Probe mix, TaqMan probe-based technology (Primer Design Ltd., Southampton, UK). Results are expressed as the relative fold compared to the control animals. For each tissue, measurements were performed in triplicate with a 96-well optical plate for both targets using 9 µL cDNA (100 ng), 1 µL Primer Perfect Probe mix, and 10 µL QuantiTect Probe PCR Master mix (Qiagen) in each 20 µL reaction. The plates were heated for 2 minutes at 50°C and 10 minutes at 95°C and then 40 cycles of 15 seconds at 94°C and 60 seconds at 60°C were applied. All data are expressed as the fold-change in expression compared to the expression in other animal groups using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical Analysis

Statistical analyses were done using Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, Ill, USA) software. The results were given as mean ± SD. One-way variance analysis was used for inter-group comparisons, and all inter-group comparisons were done with the Duncan multi-comparison test. The differences between the groups were determined using the Kruskal–Wallis test and Mann–Whitney *U* test in histopathological findings ($P < .05$ was accepted as statistically significant).

Results

Histopathologic Investigatd'ns

The general histologic architectures of the respiratory and olfactory epithelium and lamina propria in the healthy group (group without rhinosinusitis) were normal (Table 2 and Figure 1A–1B). The nasal tissues in the SA group (rhinosinusitis without any treatment) showed histopathologic changes such as inflammatory cell infiltrates, olfactory and respiratory epithelial degeneration, mucosal edema, and necrotic cells. Inflammatory cell types were both mononuclear and polymorphonuclear leukocytes. Both the epithelial tissue and lamina propria of nasal respiratory and olfactory tissues had inflammatory cells (Table 2 and Figure 1C-D). The SA+CEFA group

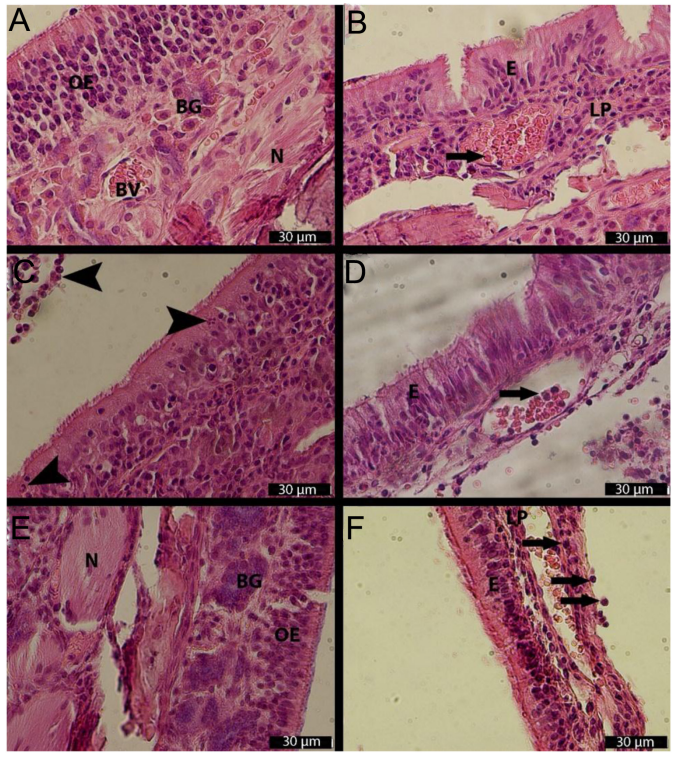

Figure 1. (A, B) Nasal tissues with normal morphology of the rats in healthy group. (A) Olfactory mucosa in healthy group showing olfactory epithelium (OE) and bowman gland (BG), blood vessel (BL), and nerve (N). (B) Respiratory mucosa in healthy group showing respiratory epithelium (E), lamina propria (LP), blood vessel (arrowhead). The general architecture of the nasal tissues in SA rats (C and D) was distorted both with inflammatory cell infiltrations (arrowhead) in olfactory epithelium (OE) and lamina proria of olfactory mucosa (C) and with inflammatory cells within blood vessel (arrowhead), in epithelium (E) and lamina proria of respiratory mucosa (D). Quite noticeable degenerative changes in both olfactory and respiratory epithelium. Histological section of the nasal tissues in SA + CEFA group (E, F) showed leukocytes infiltrations (arrowheads) in respiratory lamina propria and degenerative cellular changes at middle level. CEFA, cefazolin.

Table 2. Histopathological Score for Rat Nasal Tissues Treated with Antibiotic, Alpha-Lipoic Acid, and Combination Therapy

Group/Data	Healty Group	SA Group	SA + CEFA Group	SA + ALA 100	SA + ALA 200	SA + ALA 200 + CEFA Group
Inflammatory cell infiltrates	–	++++	++	++	++	–
Epithelial degeneration	–	++++	++	++	+	–
Necrotic cell death	–	+++	++	++	+	–
Mucosal edema	–	+++	++	+	+	–

–, none; +, minimal; ++, mild; +++, moderate; ++++, severe.
ALA, alpha-lipoic acid; CEFA, cefazolin.

(rhinosinusitis + cefazolin 50 mg/kg) showed these histopathological changes lesser than SA group and low-grade inflammatory cell infiltrates (Table 2 and Figure 1E-F).

In the SA + ALA 100 (rhinosinusitis + ALA 100 mg/kg) and SA + ALA 200 (rhinosinusitis + ALA 200 mg/kg) groups, nasal tissues had olfactory and respiratory epithelial degeneration, deteriorating vascular integrity and mucosal edema at a minimal level, decreasing in a dose-dependent manner and also had regenerative changes, increasing in a dose-dependent manner. In these groups, nasal mucosa had inflammatory cells lesser than those observed in SA group (Table 2 and Figure 2A-2B). The olfactory epithelium, lamina propria, Bowman's glands, blood vessels, and nerve bundles in olfactory mucosa and ciliated pseudostratified columnar epithelium, lamina propria in the respiratory mucosa of the SA + ALA 200 + CEFA group (rhinosinusitis plus combination therapy) had normal histological structure, when compared with the healthy group (Table 2 and Figure 2C-2D).

Polymorphonuclear leukocytes were observed in the nasal smear in the healthy group, while mononuclear leukocytes were very low (Figure 3A). In the SA group, mononuclear leukocytes were observed at a severe level in the nasal smear (Figure 3B). In the SA + CEFA group, the number of mononuclear leukocytes

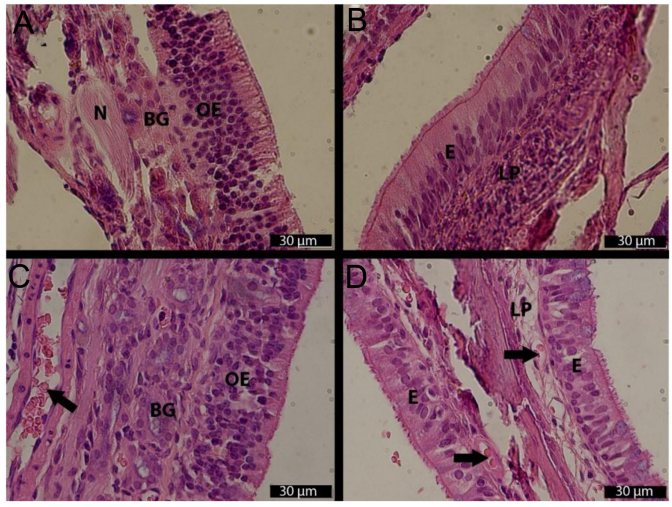

Figure 2. Histological section of the nasal tissues in SA + ALA 200 group (A, B) showed minimal degenerative changes in both olfactory and respiratory mucosa and inflammatory cell infiltrations in respiratory lamina propria (LP, B). Histological section of the nasal tissues in the SA + ALA 200 + CEFA group (C, D) showed similar patterns to the normal control. Blood vessels (arrowheads) with normal structure. ALA, alpha-lipoic acid; CEFA, cefazolin.

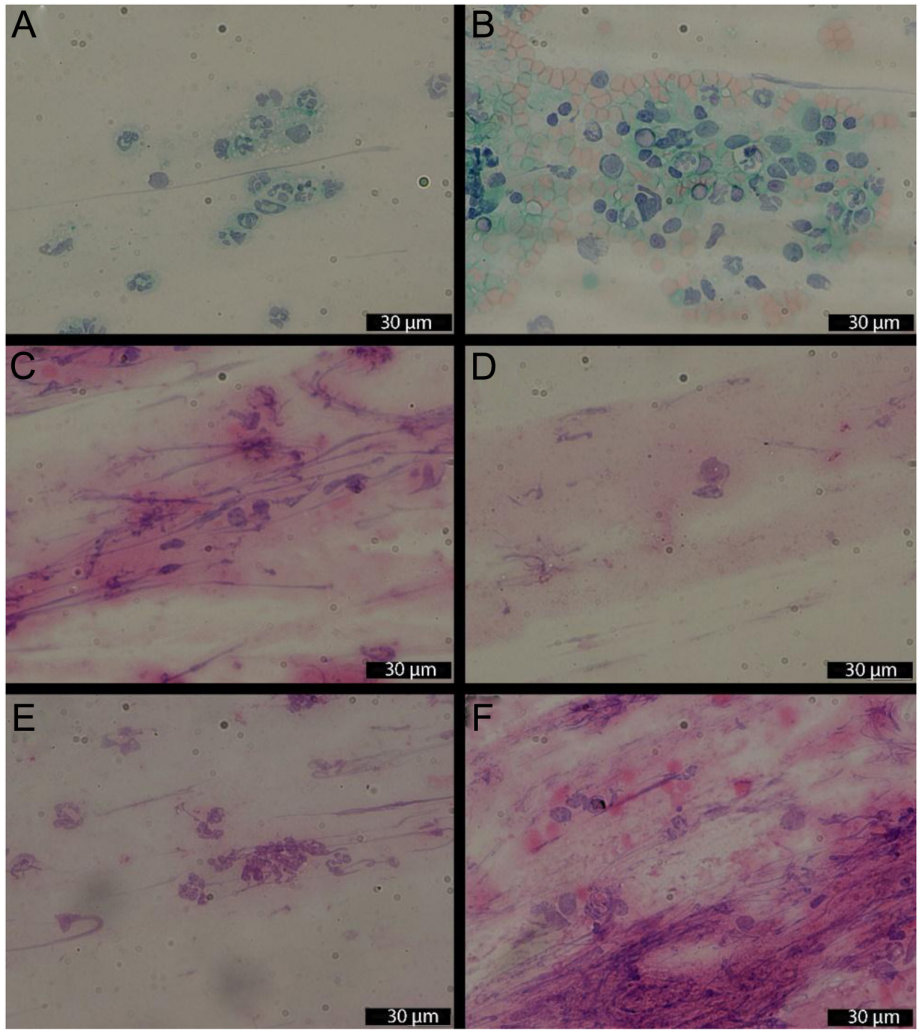

Figure 3. Hematoxylin–eosin staining of nasal smear. Histologic micrographs from healty (A), SA (B), SA + CEFA group (C), SA + ALA 200 + CEFA group (D), SA + ALA 100 (E), and SA + ALA 200 (F) groups. ALA, alpha-lipoic acid; CEFA, cefazolin.

decreased compared to the CA group (Figure 3C). The number of these cells was very low in the SA + ALA 200 + CEFA group (Figure 3D). In the SA + ALA 100 and SA + ALA 200 groups, mononuclear leukocytes relatively decreased in a dose-dependent manner compared to the SA group, and also polymorphonuclear leukocytes were observed (Figure 3E and F). The statistical graph for rat nasal tissues treated with antibiotics, ALA, and combination therapy is given in Figure 4.

Molecular Results

Whether low and high doses of ALA (100 mg/kg and 200 mg/kg, respectively) were effective in experimentally induced acute sinusitis were evaluated. The expression levels of the TNF-alpha mRNA and IL-1β mRNA were analyzed with real-time PCR in the nasal and maxillary sinus mucosa tissues of the rats. The TNF-alpha mRNA and IL-1β mRNA expressions were significantly higher in the sinusitis group when compared to the healthy group ($P < .05$). Both ALA doses significantly reduced TNF-alpha mRNA and IL-1β mRNA expression when compared to the levels in the sinusitis group ($P < .05$). IL-1 mRNA expression level in the sinusitis + ALA + CEFA group was observed to reduce to a comparable level in the healthy group ($P < .05$) (Figure 5).

Bd'chemical Results

Superoxide dismutase activity and GSH levels were found to be lower, and MDA levels were found to be significantly higher in the sino-mucosal tissue of the SA group than in the healthy control group ($P < .05$). Superoxide dismutase activity and GSH levels were found to be similar in the healthy group and the SA + ALA 200 + CEFA-treated groups. We found that all treated groups decreased MDA levels to healthy levels when compared to the SA group. When we compared groups of research, SA + ALA 200 + CEFA group was more effective in terms of increasing SOD activity and GSH and decreasing MDA levels (Figure 6-8).

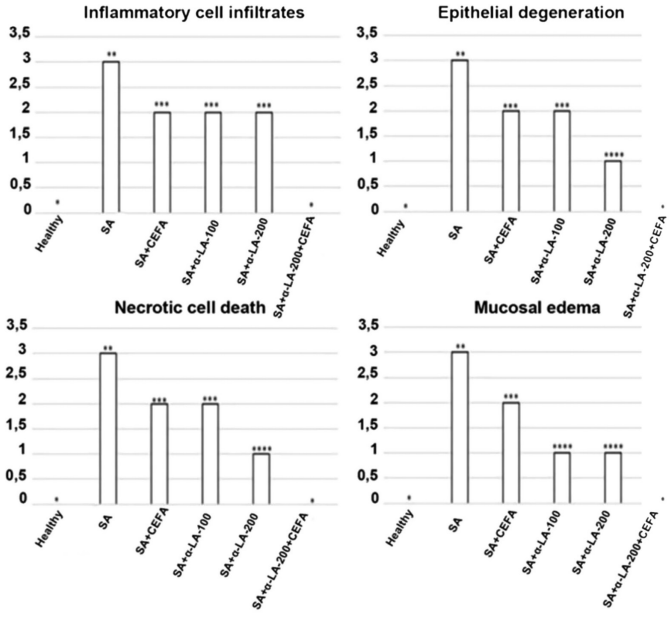

Figure 4. Statistical graphic for rat nasal tissues treated with antibiotic, alpha-lipoic acid, and combination therapy.

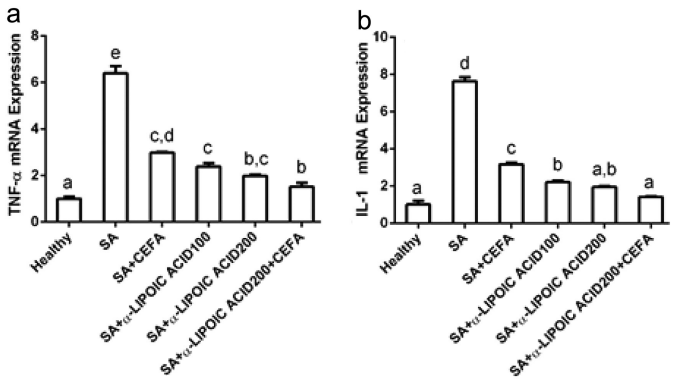

Figure 5. The influence of rhinosinusitis on TNF-α and IL-1 β activity in the maxillary sinus mucosa of the healthy rats and the treated rats with acute sinusitis. SA: rhinosinusitis, SA + CEFA (CEFA 50 mg/kg), SA + ALA 100: ALA 100 mg/kg, SA + ALA 200: ALA 200 mg/kg, SA + ALA 200 + CEFA: ALA 200 mg/kg + CEFA 50 mg/kg. Each value is mean ± SD for 6 samples in each group. Values not sharing a common superscript differ significantly at P < .05 Duncan's multiple range test (DMRT). SD, standard deviation; CEFA, cefazolin; ALA, alpha-lipoic acid.

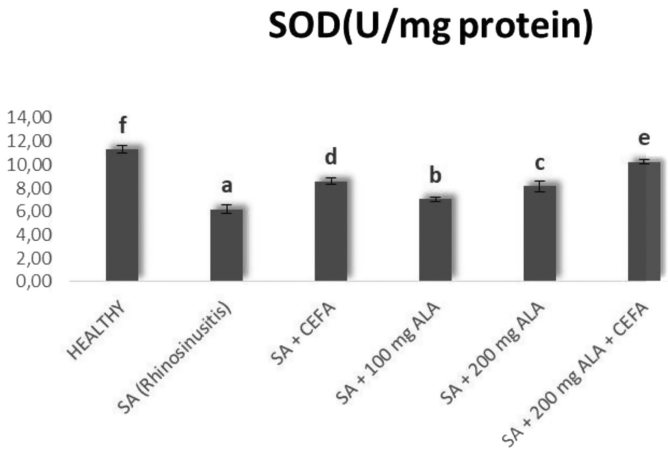

Figure 6. Superoxide dismutase levels of study groups. Groups: healthy, SA: rhinosinusitis, SA+CEFA (CEFA 50 mg/kg), SA + ALA 100, SA + ALA 200, SA + ALA 200 + CEFA. The different letters on the rods (a, b, c, etc.) indicate a statistically significant difference between groups according to the one-way analysis of variance Duncan multi-comparison test (P < .05). ALA, alpha-lipoic acid; CEFA, cefazolin.

Discussd'n

The role of inflammation in sinusitis pathophysiology should be better investigated so that better treatment strategies can be developed. The assessment of potential therapeutic benefits of anti-inflammatory agents seems to be a rational approach since inflammation seems to play an important role in sinusitis.[33] It is clear that persistent cases could be treated with a clear understanding of the pathophysiology of the disease and more effective treatments. Evidence is available indicating that some sinusitis cases are related to long-standing and excess inflammation of the sinuses rather than a simple bacterial infection.[33,34] Neutrophil granulocytes were reported to release proteolytic enzymes, and proteases could lead to tissue damage through exceeding the capacity of mucosa inhibitors.[33-35] Some researchers propose that immune reactive products could continue inflammation even after the infection had been

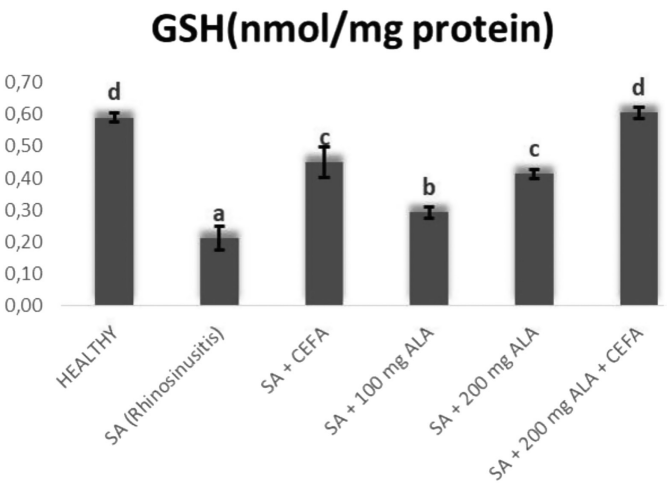

Figure 7. Glutathione (GSH) levels of the study groups. Groups: healthy, SA: rhinosinusitis, SA + CEFA (CEFA 50mg/kg), SA + ALA 100 mg/kg, SA + ALA 200 mg/kg, SA + ALA 200 mg/kg + CEFA. The different letters (a, b, c, etc.) shown on the bars in the graph indicate that there is a statistically significant difference between the groups according to the one-way analysis of variance Duncan multiple comparison test ($P < .05$). ALA, alpha-lipoic acid; CEFA, cefazolin.

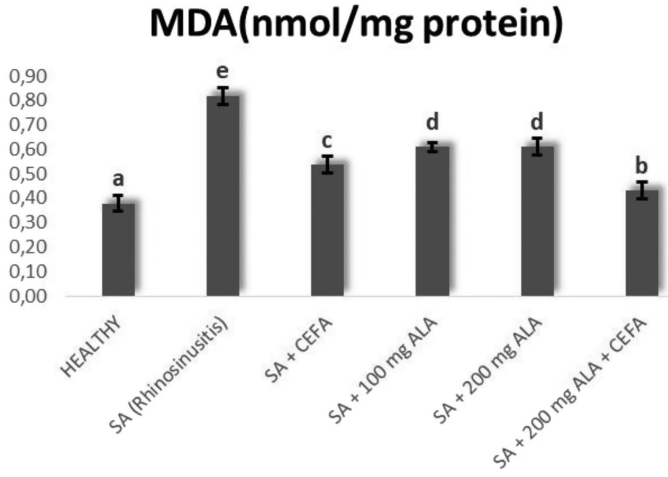

Figure 8. Malondialdehyde (MDA) levels of study groups. Groups: healthy, SA: rhinosinusitis, SA + CEFA (CEFA 50 mg/kg), SA + ALA 100 mg/kg, SA + ALA 200 mg/kg, SA + ALA 200 mg/kg + CEFA. The different letters (a, b, c, etc.) shown on the bars in the graph indicate that there is a statistically significant difference between the groups according to the one-way analysis of variance Duncan multiple comparison test ($P < .05$). ALA, alpha-lipoic acid; CEFA, cefazolin.

treated, and mucosal changes could be related to inflammatory mediators rather than the toxic effects of the microorganism.[33-36] Pathogenic microorganisms may be accepted as pro-oxidant agents since they lead to cell death and tissue damage. The organism may eliminate reactive oxygen radicals that are formed with oxidative stress response partially with specific cellular defense mechanisms. Defense mechanism products inevitably lead to cellular damage, inflammation, and characteristic oxidative stress. Free oxygen radicals protect the organism against microorganisms and lead to tissue damage through triggering inflammation during this protection. In addition, it should be kept in mind that oxidative damage observed in contagious diseases results from the inflammatory response exceeding the oxidative potential of the pathogenic agent.[37]

Severity of the bacterial infection should be further controlled with additional protective mechanisms that restrict the severity of tissue damage. Tissue damage control is a very important component of host defense mechanisms against infection. The epithelium of the upper respiratory tract represents an important physiologic barrier as the first defense barrier against the exhaled antigens and restricts disease severity without affecting pathogen load.[9] Additional protective mechanisms that restrict the severity of tissue damage like antioxidant defense mechanisms contribute to controlling the disease.

Alpha-lipoic acid is a potent antioxidant which may alter redox status and interact with thiols and other antioxidants. Alpha-lipoic acid shows its antioxidant effect through 4 different mechanisms: the ROS scavenger effect; the capacity to regenerate endogenous antioxidants like glutathione, vitamin C, and vitamin E; a metal chelating effect; and the ability to repair oxidized proteins.[13,14]

Superoxide dismutase and GSH are the main antioxidants for fighting ROS. Studies have shown that oxidative stress increases in tissues as the result of infection, and increased oxidative stress leads to tissue damage. The SOD and GSH levels were shown to decrease, and the MDA levels were shown to increase also in experiment groups in our study. Elevated MDA levels that increased in the sinusitis groups treated with only ALA decreased depending on the ALA dose; decreased antioxidant activities became close to normal. More importantly, the best improvement was obtained in the groups which were administered ALA along with the antibiotic. Many studies have revealed that ALA significantly changed MDA levels which indicate tissue damage and SOD and GSH levels in various disease groups.[13,15,38] Our study showed that ALA improved antioxidant defense mechanisms, decreased MDA levels, and more importantly, adding a potent antioxidant like ALA to standard antibiotic treatment significantly decreased tissue damage. Histopathological analyses were seen to support biochemical findings. A significant decrease in epithelial and tissue damage that developed due to sinusitis supports our hypothesis.

Pro-inflammatory cytokines like TNF-α and IL-1 play an important role in the acute inflammatory process.[37] Excess release of these cytokines leads to tissue damage. Studies have shown that elevated TNF-α and IL-1 play an important role in infectious diseases and are directly proportional to prognosis. Levels of both cytokines were elevated also in the sinusitis groups in our study. Elevated cytokine level in the groups treated with only ALA approximated to the healthy group. This may result from ALA is both a potent antioxidant and also has an anti-inflammatory effect. The elevation of free oxygen radicals is known to increase TNF-α and IL-1 expression. Antioxidant treatment or reducing oxidative stress was shown to decrease the elevated cytokine levels. Anti-inflammatory treatment was also shown to reduce the cytokine level. Alpha-lipoic acid was revealed to show a potent anti-inflammatory effect in addition to its strong antioxidant effects in previous studies. Alpha-lipoic acid treatment was also shown to significantly reduce the elevated cytokine level in sepsis.

The histopathological changes in mucosal structures are proportional to the ALA dose and the inflammatory cell infiltration

being at a minimal level. The ALA + CEFA groups having the closest histological morphology with the healthy group showed that ALA suppresses inflammation in acute sinusitis and recovers tissue damage.

Conclusd'n

Our study showed that administering a potent antioxidant agent with potential anti-inflammatory properties along with an antibacterial agent could be much more effective in reducing oxidative stress and elevating cytokine levels that result from infection in the treatment of bacterial sinusitis. The decrease in free oxygen radicals also corrected histopathological disorders. The present study showed that using ALA could be much more effective for reducing tissue damage, and these results should be supported by clinical studies.

Ethics Committee Approval: This study was approved by Ethics committee of Atatürk University, (Approvel No: 2019-4/55).

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References

1. Lanza DC, Kennedy DW. Adult rhinosinusitis defined. *Otolaryngol Head Neck Surg*. 1997;117:1-6.
2. O'Brien KL, Dowell SF, Schwartz B, Marcy SM, Phillips WR, Gerber MA. Acute sinusitis—principles of judicious use of antimicrobial agents. *Pediatrics*. 1998;101(Supplement_1):174-177. [CrossRef]
3. Paulis G. Inflammatory mechanisms and oxidative stress in prostatitis: the possible role of antioxidant therapy. *Res Rep Urol*. 2018;10:75-87. [CrossRef]
4. Wu X, Zhang Y, Chen X, Chen J, Jia M. Inflammatory immune response in rabbits with Staphylococcus aureus biofilm-associated sinusitis. *Int Forum Allergy Rhinol*. 2018;8(11):1226-1232. [CrossRef]
5. Lugrin J, Rosenblatt-Velin N, Parapanov R, Liaudet L. The role of oxidative stress during inflammatory processes. *Biol Chem*. 2014;395(2):203-230. [CrossRef]
6. Harijith A, Ebenezer DL, Natarajan V. Reactive oxygen species at the crossroads of inflammasome and inflammation. *Front Physiol*. 2014;5:352. [CrossRef]
7. Kennett EC, Chuang CY, Degendorfer G, Whitelock JM, Davies MJ. Mechanisms and consequences of oxidative damage to extracellular matrix. *Biochem Soc Trans*. 2011;39(5):1279-1287. [CrossRef]
8. Mrowicka M, Zielinska-Blizniewska H, Milonski J, Olszewski J, Majsterek I. Evaluation of oxidative DNA damage and antioxidant defense in patients with nasal polyps. *Redox Rep*. 2015;20(4):177-183. [CrossRef]
9. Tatar A, Korkmaz M, Yayla M, et al. The potential role of amlodipine on experimentally induced bacterial rhinosinusitis. *Braz J Otorhinolaryngol*. 2017;83(6):619-626. [CrossRef]
10. Birdane L, Cingi C, Muluk NB, San T, Burukoglu D. Evaluation of the efficacy of curcumin in experimentally induced acute sinusitis in rats. *Ear Nose Throat J*. 2016;95(12):E21-E27.
11. Yoruk O, Tatar A, Keles ON, Cakir A. The value of Nigella sativa in the treatment of experimentally induced rhinosinusitis. *Acta Otorhinolaryngol Ital*. 2017;37(1):32-37. [CrossRef]
12. Kalghatgi S, Spina CS, Costello JC, et al. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. *Sci Transl Med*. 2013;5(192):192ra85. [CrossRef]
13. Odabasoglu F, Halici Z, Aygun H, et al. Alpha-lipoic acid has anti-inflammatory and anti-oxidative properties: an experimental study in rats with carrageenan-induced acute and cotton pellet-induced chronic inflammations. *Br J Nutr*. 2011;105(1):31-43. [CrossRef]
14. Huong DT, Ide T. Dietary lipoic acid-dependent changes in the activity and mRNA levels of hepatic lipogenic enzymes in rats. *Br J Nutr*. 2008;100(1):79-87. [CrossRef]
15. Tatar A, Yoruk O, Unal D, Selli J, Yayla M, Halıcı Z. The effect of alpha-lipoic acid on Myringosclerosis in a rat model. *Int Adv Otol*. 2015;10(3):222-227. [CrossRef]
16. Whiteman M, Halliwell B. Protection against peroxynitrite-dependent tyrosine nitration and alpha 1-antiproteinase inactivation by ascorbic acid. A comparison with other biological antioxidants. *Free Radic Res*. 1996;25(3):275-283. [CrossRef]
17. Packer L. LAlpha-lipoic acid: a metabolic antioxidant which regulates NF-kappa B signal transduction and protects against oxidative injury. *Drug Metab Rev*. 1998;30(2):245-275. [CrossRef]
18. Kara A, Akman S, Demİrcİ T, Arabaci T, Çanakçi V, Ünal B. Influence of alpha lipoic acid on epithelial apoptosis in experimental periodontitis. *Turk J Med Sci*. 2013;43:747-755. [CrossRef]
19. Li G, Fu J, Zhao Y, Ji K, Luan T, Zang B. Alpha-lipoic acid exerts anti-inflammatory effects on lipopolysaccharide-stimulated rat mesangial cells via inhibition of nuclear factor kappa B (NF-κB) signaling pathway. *Inflammation*. 2015;38(2):510-519. [CrossRef]
20. Dietrich M, Helling N, Hilla A, et al. Early alpha-lipoic acid therapy protects from degeneration of the inner retinal layers and vision loss in an experimental autoimmune encephalomyelitis-optic neuritis model. *J Neuroinflammation*. 2018;15(1):71. [CrossRef]
21. Smirnova J, Kabin E, Jarving I, et al. Copper(I)-binding properties of de-coppering drugs for the treatment of Wilson disease. Alpha-Lipoic acid as a potential Anti-Copper agent. *Sci Rep*. 2018;8:1-9.
22. Ambrosi N, Guerrieri D, Caro F, et al. Alpha lipoic acid: a therapeutic strategy that tend to limit the action of free radicals in transplantation. *Int J Mol Sci*. 2018;19(1):102. [CrossRef]
23. Wang X, Lin H, Xu S, Jin Y, Zhang R. The clinical efficacy of epalrestat combined with alpha-lipoic acid in diabetic peripheral neuropathy: protocol for a systematic review and meta-analysis. *Med (Baltim)*. 2018;97(6):e9828. [CrossRef]
24. Sayın M, Temiz P, Var A, Temiz C. The dose-dependent neuroprotective effect of alpha-lipoic acid in experimental spinal cord injury. *Neurol Neurochir Pol*. 2013;47(4):345-351. [CrossRef]
25. Cadirci E, Altunkaynak BZ, Halici Z, et al. Alpha-lipoic acid as a potential target for the treatment of lung injury caused by cecal ligation and puncture-induced sepsis model in rats. *Shock*. 2010;33(5):479-484. [CrossRef]
26. Kaplan KA, Odabasoglu F, Halici Z, et al. Alpha-lipoic acid protects against indomethacin-induced gastric oxidative toxicity by modulating antioxidant system. *J Food Sci*. 2012;77(11):H224-H230. [CrossRef]
27. Eti CM, Vayısoğlu Y, Kardaş B, et al. Histopathologic evaluation of Ecballium elaterium applied to nasal mucosa in a rat rhinosinusitis model. *Ear Nose Throat J*. 2018;97(6):E14-E17. [CrossRef]
28. Kaya Z, Yayla M, Cinar I, et al. Effect of montelukast, a cysteinyl leukotriene receptor-1 antagonist, on a rat model of acute bacterial

sinonasal inflammation. *Am J Rhinol Allergy*. 2019;33(5):559-566. [CrossRef]
29. Uchida R, Okamoto H, Ikuta N, Terao K, Hirota T. Enantioselective pharmacokinetics of α-lipoic acid in rats. *Int J Mol Sci*. 2015;16(9):22781-22794. [CrossRef]
30. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. 1968;25(1):192-205. [CrossRef]
31. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351-358. [CrossRef]
32. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem*. 1988;34(3):497-500. [CrossRef]
33. Cable BB, Wassmuth Z, Mann EA, et al. The effect of corticosteroids in the treatment of experimental sinusitis. *Am J Rhinol*. 2000;14(4):217-222. [CrossRef]
34. Engquist S, Lundberg C, Venge P. Granulocyte proteases in human maxillary sinus secretions. *Scand J Infect Dis*. 1983;15(1):119-123. [CrossRef]
35. Olsson I, Venge P. The role of the human neutrophil in the inflammatory reaction. *Allergy*. 1980;35(1):1-13. [CrossRef]
36. Westrin K, Stierna P, Stierna P, Karlsson G, Melen I, Jannert M. *Pathophysiology of Sinusitis: Overview and Update. Aspects on Sinusitis: Diagnosis and Treatment in Adults*. Stockholm: Eli Lilly Sweden; 1995:13-22.
37. Romero Alvira D, Guerrero Navarro L, Gotor Lázaro MA, Roche Collado E. Oxidative stress and infectious pathology. *An Med Interna*. 1995;12(3):139-149.
38. Ahn SK, Jeon SY, Khalmuratov R, et al. Rat model of staphylococcal enterotoxin B-induced rhinosinusitis. *Clin Exp Otorhinolaryngol*. 2008;1(1):24-28. [CrossRef]