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# The effect of Escherichia Coli induction on superoxide dismutase (SOD) and Malondialdehyde (MDA) levels in acute rhinosinusitis white rats models



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## ABSTRACT

**Background:** Acute Rhinosinusitis is an inflammation of the paranasal sinuses and the nasal cavity lasting no longer than 6 weeks. One of the etiologies is the gram-negative bacteria, the lipopolysaccharide layer containing bacteria *Escherichia coli*. Lipopolysaccharide was the leading cause of inflammatory mechanisms, and subsequent Reactive Oxygen Species production was thought of as the agent that injure the cells through the oxidative stress. This study aimed to determine the effect of E-coli induction on the ratio of SOD and MDA levels in acute rhinosinusitis white rats model.

**Methods:** This study used an experimental post-test only control group design with white rats strain that met the inclusion and exclusion criteria divided into two groups. The study includes 8 rats as control groups and 32 rats in the treatment group. Treatment includes induction of rhinosinusitis by E-coli. SOD and MDA levels measured

from retro-orbital venous blood samples were measured as marker oxidative stress on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days. Data were analyzed using SPSS version 22 for Windows.

**Results:** There were significant differences in SOD and MDA levels from day 7 to day 28 between the control and the intervention groups ( $p < 0.001$ ). The mean SOD levels on days 7, 14, 21, and 28, were lower than those of the control group. The MDA levels from day 7 to day 28 of the intervention group was significantly higher than that of the control group ( $p < 0.001$ ). Spearman Correlation test obtained a significant correlation between SOD and MDA levels ( $r = -0.604$ ;  $p < 0.001$  on day 7 and  $r = -0.453$ ;  $p < 0.003$ ).

**Conclusion:** There is a significant correlation between the effect of induced *E coli* on SOD and MDA levels in acute rhinosinusitis white rats models.

**Keywords:** Acute Rhinosinusitis, *Escherichia coli*, SOD, MDA.

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## INTRODUCTION

Acute rhinosinusitis is defined as an inflammatory process of the nasal mucosa and the paranasal sinuses, that is caused by viruses or bacteria and has a duration of less than 4 weeks.<sup>1,2</sup> Rhinosinusitis based on a European position paper on rhinosinusitis and nasal polyp (EPOS) is defined as two or more symptoms, in which one of them should be either nasal blockage/ obstruction/ congestion or runny (anterior/ posterior nasal secret), facial pain/ depressed sensation, decrease/ loss of smell.<sup>1,2</sup> It is a sudden onset, it lasts no more than 12 weeks with free symptom interval if it is recurrent. This condition not only causes physical problems such as nasal congestion but also affects the daily psychological and socio-economic welfare that ultimately the quality of life of the sufferers.<sup>3-5</sup>

Acute rhinosinusitis is based on the rhinitis inflammatory mechanisms. The cause could be gram-positive and gram-negative bacteria.<sup>4,5</sup> The pathogenic effect of the bacteria related to the

components of the cell wall, especially the lipopolysaccharide layer (LPS).<sup>5,6</sup> LPS is an important endotoxin and antigen-specific group. LPS exposure lead to increased production of pro-inflammatory mediators such as TLRs, TNF- $\alpha$ , NF- $\kappa$ B, IL-6, IL-8 as a body defense mechanism against foreign substances which have positive and negative impacts.<sup>6,7</sup>

In acute rhinosinusitis, progressive deterioration of nose and paranasal sinuses functions will induce some biological and clinical dysfunctions.<sup>7,8</sup> These include changes in cellular energy metabolism and nitrogen input/output, increased synthesis of inflammatory mediators, and oxidative stress. Oxidative stress and inflammation promote injury through damage in the molecular component.<sup>7,8</sup> Inflammation in the nose and paranasal sinuses is initiated by the free radical generated from intracellular or extracellular oxygen derivatives and its inflammatory response. Free radicals such as

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superoxide and hydroxyl molecules easily interact with molecular components in cells in the nose and the paranasal sinuses.<sup>6-8</sup> These radical immune responses stimulate the release of additional pro-inflammatory signals resulting in the formation of other free radicals and/or reactive oxygen species (ROS) and progressive damages of the molecular components.<sup>9</sup>

The body will respond increased formation of free radicals (ROS) by producing superoxide dismutase (SOD) through the activation of Nuclear Respiratory Factor 2 (Nrf2) which then it is translocated to the nucleus that binds to the Antioxidant Respond Element (ARE).<sup>7-9</sup> Therefore it can regulate the expression of the enzymatic antioxidant gene.<sup>10,11</sup> Superoxide dismutase (SOD) is a metalloenzyme antioxidant. It prevents cellular damage by converting the anion of superoxide into another less harmful component, namely hydrogen peroxide.<sup>10,11</sup> In mitochondria, this hydrogen peroxide will be detoxified by catalyst enzyme into H<sub>2</sub>O and O<sub>2</sub> by glutathione peroxidase enzyme if it diffused into the cytosol.<sup>11,12</sup> Although human body cells equipped with in-house mechanisms to control ROS, imbalances can occur when ROS levels increase. This appears when the SOD enzymes are unable to neutralize the excessive ROS. This condition can lead to cell damage.<sup>10,12</sup>

In order to assess the oxidative stress that occurs in acute rhinosinusitis, we use the Malondialdehyde (MDA) parameter as well as a high level of MDA due to the effect of increased ROS. Reactive oxygen species production can be measured with various measurements; one of them is the Thiobarbituric Acid Reactive Substance (TBARS) test.<sup>11</sup> This study aims to evaluate the effect of *Escherichia Coli* induction on superoxide dismutase (SOD) and Malondialdehyde (MDA) levels in acute rhinosinusitis white rats models.

## METHODS

An experimental post-test only study with a control group design was conducted to analyze the effect of *Escherichia coli's* lipopolysaccharide induction on the immune system. The study subjects were 40 White Sprague Dawley rats obtained from the Faculty of Veterinary Medicine, Universitas Gajah Mada, Yogyakarta, Indonesia. We used these white rats because they are commonly used for biomedical study, relatively large. Despite the different sinus anatomy of mice from that of human beings, the respiratory epithelium is similar so that epithelial remodeling inflammatory cell infiltration, as well as collagen deposition of these mice can be evaluated

during experimental study.<sup>13</sup> We included active healthy Sprague Dawley (SD) rats with glowing eyes and bright fur, aged between 3 and 4 months weighing 200 -300 grams. The SD rats which died during the study period were excluded from the study.

All these SD rats were acclimated for 7 days, and then they were grouped into control (n=8) and intervention (n=32). Each rat in the intervention group received E-coli Lipopolysaccharide (LPS) with a dose of 0,1 ml containing  $1.5 \times 10^9$  CFU/ml by intranasal and intraperitoneal injections to induce acute rhinosinusitis. This procedure was done in animal model study center Pusat Antar Universitas (PAU) Universitas Gadjah Mada in Yogyakarta.

The SOD and MDA levels of all the rats were measured with Enzyme-Linked Immunosorbent Assay (ELISA) on days 7, 14, 21 and 28 in the Biomedical Laboratory of Medical Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia. The Data were presented in mean  $\pm$  SD and analyzed with SPSS 22 for windows. Mann-Whitney and Spearman tests were used in this study due to data were not normally distributed.

## RESULTS

In this study, the levels of SOD and MDA in both groups were measured on days 7, 14, 21, 28. The SOD levels of both groups on those days showed a downward trend over time. However, the SOD level of the intervention groups was lower than that of control groups ( $p < 0.001$ ). The significant decline of SOD started on day 21 for both the control and the intervention groups and reached the lowest level on day 28 ( $p < 0.001$ ) (Table 1).

Our MDA measurement obtained the increasing level in both groups, but the significant increase was observed in LPS induced rats ( $p < 0.001$ ). The increase reached the highest level on day 28, from  $2.27 \pm 0.40$  on day 7 to  $9.06 \pm 0.38$  on day 28 in the intervention group and from  $0.76 \pm 0.21$  on day 7 to  $1.27 \pm 0.17$  on day 28 in the control group (Table 1).

In the Mann-Whitney test on MDA levels, since the p-value  $< 0.05$  on day 7 to day 28, therefore there is a significant difference in MDA levels on day 7 to day 28 in the control group and treatment group of *Escherichia coli* induction in acute rhinosinusitis white rats model. Spearman correlation test of SOD and MDA revealed a significant correlation between SOD and MDA in LPS-induced rats. This strongest correlation was found on day 7 (correlation coefficient + - 0.604;  $p < 0.001$ ) (Table 2)

**Table 1**

Measurement	Group		Mean Difference	p
	Control (n=8)	Treatment (n=32)		
<b>SOD (mean±SD)</b>				
Day 7	85.61±3.71	69.70±4.54	15.91±0.83	0.001
Day 14	83.33±3.14	56.16±4.35	27.17±1.21	0.001
Day 21	78.22±3.88	33.38±4.66	44.84±0.78	0.001
Day 28	75.43±4.71	20.97±5.49	54.56±0.78	0.001
<b>MDA (mean±SD)</b>				
Day 7	0.76±0.21	2.27±0.40	1.51±0.19	0.001
Day 14	0.88±0.17	4.43±0.40	3.55±0.23	0.001
Day 21	0.99±0.17	7.31±0.36	6.32±0.19	0.001
Day 28	1.27±0.17	9.06±0.38	7.79±0.21	0.001

SOD: superoxide dismutase; MDA: malondialdehyde; p:Mann-Whitney test was considered significant if less than 0.05.

**Table 2 Correlation Test of SOD and MDA**

Variables	Correlation Coefficient	P-value
SOD Day 7 – MDA Day 7	-0.604	0.001
SOD Day 14 – MDA Day14	-0.597	0.001
SOD Day 21 – MDA Day 21	-0.507	0.001
SOD Day 28 – MDA Day 28	-0.453	0.003

## DISCUSSION

Reactive Oxygen Species (ROS) are produced by cellular metabolism. However, the overproduction of ROS and its derivatives may poorly affect health. Among ROS, the superoxide anion plays a vital role in inflammation. The enzyme SOD neutralizes superoxide anion by converting it into hydrogen peroxide so that it prevents the formation of aggressive compounds like peroxynitrite and hydroxyl radical.<sup>14</sup> One primary indication of acute kidney injury progression is Oxidative stress. This is characterized by an aggregation of increased ROS and damaged antioxidant capability. LPS can induce ROS production and inhibit the antioxidant defense process contributing to oxidative kidney damage. In general, free radical production is abolished via intracellular antioxidant enzymes like SOD.<sup>15</sup>

In our study, the SOD levels of both control and intervention groups showed a downward trend. The mean SOD level at each measurement was lower in the intervention group than that of in control group. A study by Mittal et al reported that SOD is one of antioxidant in the body which can be imbalanced when ROS is excessive due to continuous inflammation. Hence, SOD could not neutralize ROS, leading to cell damage.<sup>8</sup>

In contrast to SOD, we found that MDA levels in the control and the intervention groups demonstrated an increasing trend over time. However, the intervention group had a higher MDA level than that of the control group. Our finding is line with the study by Tasdemir et al., which investigated the effect of E-Coli induction in a dose of 0,5 ml with the concentration  $2 \times 10^8$  an animal model revealing E- Coli induction resulted in increased MDA level.<sup>16</sup> Another study by Chen et al. and other studies found that the MDA level was elevated significantly while SOD activity and Glutathione (GSH) content were decreased remarkably in LPS-induced acute kidney injury model.<sup>15-17</sup>

## CONCLUSION

Escherichia Coli induction administered to SD rats affects on SOD and MDA levels. Superoxide Dismutase (SOD) level significantly correlates with the MDA level in acute rhinosinusitis SD rat models.

## FUNDING DISCLOSURE

The authors self-funded the research.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of the interest in conducting and reporting the research.

## ETHICS CONSIDERATION

This study was approved by the ethical clearance committee of Medical Faculty Sebelas Maret University/ Dr. Moewardi Hospital Surakarta.

## AUTHORS CONTRIBUTION

The authors contributed equally during the research and manuscript writing.

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