

Biochemical parameters and their effect on lipopolysaccharides in the small intestine of male albino rats

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Abstract:

Background: Stimulation of immune cells through bacterial infection and endotoxin (LPS) leads to an increase in the production of reactive oxygen species (ROS), which in turn activates antioxidants, namely Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px), in order to reduce the harmful effects of free radicals and maintain cellular homeostasis. Meanwhile, excessive ROS production leads to a raise in lipid peroxidation, with Malondialdehyde (MDA) being one of its most important products and a direct biomarker of oxidative damage in cell membranes. **Aim:** this study aimed to compare the effects of selected Gram-positive bacteria, Gram-negative bacteria, and purified lipopolysaccharide on serum oxidative stress biomarkers (T-SOD, GPX, and MDA) in male albino rats. **Methodology:** (12 rats/group). Animals were given a single administration of distilled water (control), *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and LPS at a dose of 2.5 or 5 µg/kg. A blood sample was obtained on day 2, 4 and 6 after treatment. T-SOD, GSH-Px, and MDA determinations were performed using specific commercial ELISA kits. Statistical analysis based on one way ANOVA followed by LSD post hoc test was performed and significance level taken at $P < 0.05$ **Results:** stress biomarkers. In the *Staphylococcus aureus*, *Klebsiella pneumoniae* and high-dose LPS groups, significant alterations of T-SOD activity were observed but compared with the lowest value in the *Klebsiella pneumoniae* group at this time. GSH-Px activity showed significant differences



mostly in *Staphylococcus aureus*, *Salmonella typhimurium* and *Klebsiella pneumoniae* groups while LPS administration resulted a relatively consistent enzyme activity. In this manner, this observation demonstrates an increase in the MDA concentrations after exposure to bacteria and endotoxin, (most accentuate for strains of *Streptococcus pyogenes* as well), thereby enhancing lipid peroxidation and possibly oxidative damage. In general, Gram-negative bacteria and LPS triggered greater oxidative stress responses than controls but the relative magnitude and timing of the response differed among treatments.

Conclusions: Exposure to bacterial infection and lipopolysaccharide led to significant oxidative stress in terms of T-SOD, GSH-Px, and MDA concentrations. The initial increase in antioxidant enzymes by presumably compensating against increased ROS generation, along with continuous elevation of MDA mediate aggravation of lipid peroxidation and progressive oxidative tissue damage. The present findings indicate that oxidative stress biomarkers are reliable parameters for the monitoring of inflammation induced by bacteria and endotoxin in experimental animals.

Key word: Lipopolysaccharides, Malondialdehyde, Glutathione peroxidase, Rat model, *Staphylococcus aureus*, *Salmonella typhimurium*.

Introduction:

Throughout the different stages of an individual's life, the composition of the gut microbiota varies between individuals; however, a healthy human gut maintains an equilibrium between Gram-negative & positive bacteria, with a predominance of Gram-responsive bacteria [1]. The rate of Gram-resistant bacteria increases significantly in inflamed gut, bring about an increased concentration of lipopolysaccharide (LPS). Although lipopolysaccharides are generally considered harmless in the gut and may even offer protection against epithelial damage, some studies have suggested they may contribute to intestinal inflammation, such as enteritis. This points to a physiological clarification due to significance of keeping a stability between gram-resistant & responsive bacteria in the gut to preserve intestinal internal balance [2]. Lipopolysaccharide (LPS) is the principal element of the outer membrane of Gram-negative bacteria and commonly referred to as endotoxin, where it is recognized by the Toll-4 receptor (TLR4), which in



turn stimulates the production of multi-effect inflammatory mediators [3,4]. Numerous factors, such as medications (e.g., antibiotic use), infections, diet, and exposure to toxic chemicals, can significantly alter the composition of the gut microbiota. An increase in gram-negative bacteria in the gut can bring about elevated levels of lipopolysaccharides (LPS), capable of subsequently conduce to the development and worsening of enteritis [5].

Salmonella and *Klebsiella* are Gram-negative bacteria that exhibit a number of virulence factors, including a thick polysaccharide capsule. This capsule protects the bacteria from phagocytosis by polymorphonuclear leukocytes and is believed to prevent bacterial killing by bactericidal serum factors [6,7]. *Streptococcus* and *Staphylococcus*, which are Gram-positive bacteria, cause serious human diseases through a combination of surface virulence factors and exotoxin production [8]. One of the most effective toxins created by bacteria is the superantigen, which represents non-glycosylated proteins [9]. Superantigens synthesized by *Staphylococcus aureus* comprise enterococcal SE toxins and toxic shock syndrome toxin-1 (TSST-1), whereas those generated by *Streptococcus pyogenes* include streptococcal A and C toxins (SPEA and SPEC) and streptococcal mitotic-stimulating toxin Z (SMEZ). The metabolites of bacteria are qualified to inducing an immense cellular immune response that can trigger fatal toxic shock [9,10].

Malondialdehyde is a secondary product of polyunsaturated fatty acids. Measuring MDA levels is serves as an indicator in many diseases, both within a living organism and in controlled environment. MDA is also act in the diagnosis of diverse situations such as hypertension, diabetes, heart failure, cancer, among others [11]. Lipid peroxidation is the process of electron removal from lipids by free radicals. This mechanism generally impacts polyunsaturated fatty acids due to their numerous bonds containing active hydrogen. Their decomposition results in a variety of carbonyl compounds, with maliondaledehyde (MDA) being the most common [12,13]. This compound targets the C-C double bond in polyunsaturated fatty acids, weakening the C-H bond and producing a free radical capable of binding a hydrogen atom. This liquid free radical is then oxidized to produce a peroxy radical. This peroxy radical reacts with other polyunsaturated fatty acids. This process can continue sequentially [14].



Glutathione peroxidase (GPX) represents a family of antioxidant enzymes. It is a key class of mammalian selenoenzymes and belongs to the same class of heme-free thiol peroxidases as other peroxidases. It catalyzes the reduction of hydrogen peroxide (H_2O_2) or organic hydroperoxides to water or corresponding alcohols, thus reducing their toxicity [15]. In mammals, the enzyme glutathione peroxidase (GPX) interacts with superoxide dismutase and catalase to form an enzymatic antioxidant system that reduces reactive oxygen species (ROS) and limits their toxicity. For decades, GPX has been commonly used as a reducing agent in catalyzing the reduction of organic hydrogen peroxide (H_2O_2) to water or corresponding alcohols, respectively [16]. Reactive oxygen species (ROS) is an umbrella concept that includes oxygen radicals and non-radical compounds for instance hydrogen peroxide (H_2O_2), hydroxyl radicals, lipid radicals, and lipid (phospholipid) peroxides [17]. Reactive oxygen species have long been considered harmful substances, as their excessive accumulation leads to the discharge of inflammatory factors, enhances the bonding of these factors to endothelial cells, stimulates programmed cell death, and even causes cell death [18]. The glutathione peroxidase (GPX) family of enzymes is a key component in protecting cells from oxidative stress. Generally, GPX plays its role in resisting oxidative stress by catalyzing the reduction of hydrogen peroxide and organic lipid peroxides to water and alcohols [19].

Superoxide dismutase (SOD) is an enzyme that inhibits the body's bio-oxidant complex, which effectively responds to cellular oxidative stress, lipid metabolism, inflammation, and oxidation. It maintains a dynamic equilibrium between the production and removal of bio-oxidants in the body, preventing the toxic effects of unattached radicals. It has also demonstrated efficacy in combating tumors, radiation, and aging. Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) collectively form the body's active deoxygenation mechanism, which act a crucial role in the elimination and depletion of superoxide radicals ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet\text{OH}$). Extracellular superoxide dismutase activity reinforces the superoxide to hydroxy peroxide disequilibrium reaction. Furthermore, superoxide dismutase proteins are characterized by their capacity to link with cell surface proteoglycans and components of the extracellular matrix at a positively charged carbon



axial zone (the extracellular matrix-binding region). The performance of superoxide dismutase enzymes renders them increasingly employed in pharmaceutical, food, and skincare products. They have vital activities specifically regulating redox reactions in the body, removal free radicals, observation disease progression, and acting as antioxidants [20].

The enzyme superoxide dismutase (SOD) captures free oxygen radicals via redox cycles at an elevated reaction frequency, thanks to the conversion metal ions existing at the active site. SOD hydrolyzes $O_2^{\bullet-}$ to H_2O_2 , releasing molecular oxygen [21]. In the course of removing free radicals, SOD requires cofactors such as iron, manganese, or copper and zinc to achieve peak catalytic activity in the metabolism of toxic intermediates. The metal interaction site is situated between the two domains of SOD, and the lateral chains incorporate; aspartate, histamine, and histidine [22]. These cofactors have a tendency to grant electrons to $O_2^{\bullet-}$ and renew them through catalysis. Superoxide dismutase (SOD) also contributes to protecting the cell membrane and DNA from impairment caused by free oxygen radicals through the reaction of superoxide free radicals both inside and outside the cell (23). Superoxide dismutase (SOD) enzymes play a crucial role in balancing the concentration of biological oxidants, thus protecting all aerobic organisms from oxidative damage caused by these oxidants. Superoxide dismutase is one of the most potent antioxidant enzymes, catalyzing the conversion of oxygen (O_2) to hydrogen peroxide (H_2O_2) to regulate signaling in the body [24,25].

Methods:

Study design

A total of 84 experimental animals were allocated into seven categories, with 12 males in each category, as outlined below:

Group 1 (G1): Negative untreated group, administered tap water daily for 30 days.

Group 2 (G2): Animals administered a bacterial suspension of *Staphylococcus aureus* at a dose of 300 μ L, given once only.



Group 3 (G3): Animals administered a bacterial suspension of *Streptococcus pyogenes* at a dose of 300 μ L, given once only.

Group 4 (G4): Animals administered a bacterial suspension of *Salmonella typhimurium* at a dose of 300 μ L, given once only.

Group 5 (G5): Animals administered a bacterial suspension of *Klebsiella pneumoniae* at a dose of 300 μ L, given once only.

Group 6 (G6): Animals administered a diluted concentration of LPS (2.5 μ g/kg).

Group 7 (G7): Animals administered a concentrated dose of LPS (5 μ g/kg).

Each rat was inoculated with 300 microliters of a bacterial solution whose cell density was determined according to the McFarland Turbidity Standards opacity (No. 0.5). This solution included the following bacteria: *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella Typhimurium*, and *Klebsiella pneumoniae*.

A 20 mg LPS solution was prepared by mixing it with 36 ml of normal saline. 24 ml of this total solution was used to inoculate the first 12 rats with the concentrated solution at a rate of 2 ml per rat. The remaining 12 ml of the highly concentrated was mixed with 12 ml of normal saline to produce 24 ml of diluted solution. 2 ml of this diluted solution was then administered to each of the 12 rats in the second group.

Sample Collection and Analyses:

At specified time points, the animals were anesthetized using a piece of cotton soaked with an anesthetic agent (chloroform), which was placed inside a tightly sealed transparent container. The animal was quickly introduced into the container, and the lid was securely closed. After confirming adequate anesthesia, the animal was removed, and blood was collected directly from the heart via cardiac puncture using a sterile 5 mL syringe to obtain the maximum possible volume of blood. The blood samples were immediately transferred into sterile test tubes, namely EDTA tubes and gel tubes. The gel tubes were then centrifuged at 3000 rpm for 15 minutes to obtain serum. Subsequently, the serum was transferred into clean, dry, and labeled Eppendorf tubes and stored in a refrigerator at -20°C . The EDTA tubes were also preserved until further analysis.



Parameters Used for the Analysis of Study Samples:

Determination of MDA, GPX, T-SOD using ELISA:

The ELISA test is characterized by high sensitivity and can be used in the diagnosis of various infectious and immunological diseases, as well as in molecular research. The assay is performed according to the following steps:

Dilution of Standards:

Standards are diluted using five small tubes. incorporate 150 μL of serial diluent per tube following this, add 300 μL of SanLong standard to the first tube and blend gently. Move 300 μL from tube1 to the tube2 and mix. Next, transfer 150 μL from the second tube to the third tube and mix. Then, move 150 μL from the tube number three to tube number four and mix. Finally, transfer 150 μL from the tube4 to the tube5 and shake well.

Sample Loading:

In the Micro ELISA strip plate, leave five wells empty as blank controls and add 40 μL of assay buffer per well. Next, introduce 10 μL of the sample to each of the remaining wells. The samples should be added to the bottom of the wells without touching the walls. Mix gently.

Incubation: Maintain at 37 °C at 30 minutes upon securing the adhesive film.

Wash Buffer Preparation: Reconstitute the concentrated wash solution with deionized water (30-fold for 96T and 20-fold for 48T).

Washing:

Cautiously take off the sealing film, aspirate the contents, moreover fill the wells alongside wash solution. After allowing it to stand for 30 seconds, discard the wash solution. Repeat the washing step five times.

Addition of HRP-Conjugate: Add 50 μL of HRP-conjugate reagent to each well except the blank control wells.

Incubation: Repeat the incubation step as described in the third stage.



Washing: Replicate the washing step as detailed in the fifth part.

Chromogenic Reaction:

Introduce 50 μL of Chromogen Solution A and 50 μL of Chromogen Solution B to each well. Agitate at low speed and incubate at 37 °C for 15 minutes. Avoid exposure to light throughout this stage.

Reaction Termination:

Add 50 μL of stop solution to each well to terminate the reaction. The color should change from blue to yellow.

Measurement:

Measure the optical density (OD) at 450 nm using a microtiter plate reader.

The OD value of the blank control well is tare. The analysis should be performed within 15 minutes after post the cease solution.

Biostatistical Analysis:

To statistically evaluate the results, single factor ANOVA was conducted, succeeded by the Least Significant Difference (LSD) examination for multiple comparisons at significance levels of ($P \leq 0.01$) and ($P \leq 0.05$), using SPSS software version 24.

Result:

The results in Table (1) showed no notable differences in the control group (G1) across the different time periods ($P=0.9950$). In contrast, marked differences were recorded in the *Staphylococcus* (G2) ($P=0.0231$), with a marked decrease in activity on day 4 compared to days 2 and 6. The *Streptococcus* (G3) did not show significant differences ($P=0.1152$), although there was some fluctuation in values between the time periods. Similarly, the *Salmonella* group (G4) did not show significant differences ($P=0.0628$), with a gradual decrease on day 6. However, the *Klebsiella* group (G5) showed highly significant differences ($P=0.00034$), with a decrease in activity on day 4 compared to the other periods. While the LPS group at a concentration of 2.5 $\mu\text{g/kg}$ (G6) showed no significant differences



($P=0.4223$). in contrast, the LPS group at a concentration of 5 $\mu\text{g/kg}$ (G7) showed significant differences ($P=0.03428$), with activity being high on days 2 and 4 and then decreasing significantly on day 6. The comparison between the groups also showed highly significant differences on days 2 and 4 ($P=0.00084$ and $P=0.00184$, correspondingly), while no substantial variations were observed on day 6 ($P=0.21646$).

Table (1) Temporal dynamics of serum T-SOD pg/ml levels following exposure to Gram-resistant & responsive bacteria and lipopolysaccharide (LPS).

Groups	Time (days) after dosing (mean $\pm$ SD)			P value	LSD
	Two	Four	Six		
G1 Control	1264.7 $\pm$ 401.3 ^b A	1236.9 $\pm$ 386.8 ^b A	1250.8 $\pm$ 394.4 ^a A	0.9950	NS
G2 Staphylococcus	1088.9 $\pm$ 151.7 ^b B	469.5 $\pm$ 195.6 ^a A	820.9 $\pm$ 367.8 ^a AB	0.0231*	409.48
G3 Streptococcus	551.7 $\pm$ 56.7 ^a A	1235.6 $\pm$ 700.6 ^b A	555.5 $\pm$ 420.4 ^a A	0.1152	NS
G4 Salmonella	1445.4 $\pm$ 341.6 ^{bc} A	1314.0 $\pm$ 90.5 ^b A	1047.2 $\pm$ 65.6 ^a A	0.0628	NS
G5 Klebsiella	1255.8 $\pm$ 32.1 ^b B	641.4 $\pm$ 72.9 ^a A	1068.7 $\pm$ 218.9 ^a B	0.00034	215.22
G6 LPS(2.5 $\mu\text{g/Kg}$)	1396.2 $\pm$ 529.8 ^{bc} A	1420.2 $\pm$ 598.4 ^b A	1006.8 $\pm$ 202.8 ^a A	0.4223	NS
G7 LPS(5 $\mu\text{g/Kg}$)	1744.2 $\pm$ 212.6 ^c B	1770.3 $\pm$ 162.0 ^b B	1040.9 $\pm$ 580.9 ^a A	0.03428*	590.65
P value	0.00084**	0.00184**	0.21646		
LSD	441.53	576.74	NS		

NS means no significant differences * means significant differences ** means high significant differences

The similarity of the lowercase letters vertically indicates that there are no significant differences between the seven dosing groups.

Different lowercase letters arranged vertically indicate significant differences between the seven dosing groups.

The horizontally identical capital letters indicate that there are no significant differences between the three groups according to time.

Different horizontal capital letters indicate significant differences between the three groups according to time.

Table (2) shows no significant differences in the control group (G1) across the time periods ($P=0.8230$). However, the *Staphylococcus* group (G2) showed significant differences ($P=0.0121$), with activity being high on day 2 and decreasing on days 4 and 6. The *Streptococcus* group (G3) did not show significant differences ($P=0.4523$). The *Salmonella* group (G4), however, showed highly significant differences ($P=0.00008$), with activity decreasing on day 4 and increasing again on day 6. The *Klebsiella* group (G5) also showed significant differences ($P=0.0387$), with activity increasing on days 4 and 6 compared to day 2. In contrast, the LPS groups (G6 and G7) did not discernible distinction ($P=0.71964$ and $P=0.97046$, respectively). The comparison between the groups showed



significant differences only by the second day ($P=0.03257$), while no substantial variations were observed on days 4 and 6.

Table (2) Temporal dynamics of serum GSH-Px pg/ml levels following exposure to Gram-resistant & responsive bacteria and lipopolysaccharide (LPS).

Groups	Time (days) after dosing (mean $\pm$ SD)			P value	LSD
	Two	Four	Six		
G1 Control	345.5 $\pm$ 312.0 ^a A	460.8 $\pm$ 182.4 ^a A	403.15 $\pm$ 264.0 ^a A	0.8230	NS
G2 Staphylococcus	726.3 $\pm$ 1.15 ^c B	322.05 $\pm$ 233.0 ^a A	351.3 $\pm$ 164.4 ^a A	0.0121*	263.42
G3 Streptococcus	432.6 $\pm$ 131.9 ^{ab} A	408.1 $\pm$ 338.5 ^a A	599.5 $\pm$ 134.3 ^a A	0.4523	NS
G4 Salmonella	678.3 $\pm$ 40.9 ^{bc} B	373.7 $\pm$ 54.1 ^a A	714.3 $\pm$ 92.1 ^a B	0.00008**	105.71
G5 Klebsiella	264.5 $\pm$ 96.3 ^a A	518.2 $\pm$ 168.0 ^a B	584.9 $\pm$ 186.9 ^a B	0.0387*	248.04
G6 LPS(2.5 μ g/Kg)	448.3 $\pm$ 217.4 ^{ab} A	343.4 $\pm$ 230.0 ^a A	458.7 $\pm$ 206.5 ^a A	0.71964	NS
G7 LPS(5 μ g/Kg)	446.2 $\pm$ 316.1 ^{ab} A	476.9 $\pm$ 113.2 ^a A	444.1 $\pm$ 148.7 ^a A	0.97046	NS
P value	0.03257*	0.80065	0.10036		
LSD	290.37	NS	NS		

NS means no significant differences * means significant differences ** means high significant differences

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Different horizontal capital letters indicate significant differences between the three groups according to time.

Table (3) shows significant differences in the (G1) ($P=0.0201$), with values increasing on day 4 compared to the other periods. Distinct variations were also recorded in the *Staphylococcus* (G2) ($P=0.0135$), with a gradual increase in level until day 6. The *Streptococcus* (G3) showed highly significant differences ($P=0.000031$), with MDA levels rising significantly on day 6 compared to the previous days. In contrast, the *Salmonella* (G4) and *Klebsiella* (G5) ($P=0.4425$) did not show significant differences ($P=0.0537$). The LPS group at a concentration of 2.5 μ g/kg (G6) showed significant differences ($P=0.04367$), with levels gradually decreasing over time. The LPS group at a concentration of 5 μ g/kg (G7) showed no significant differences ($P=0.2087$). When comparing the groups, highly discernible distinctions were found during the second day ($P=0.0000234$)



and significant differences were found on day 4 ($P=0.00419$), while no notable differences were observed on day 6 ($P=0.443902$).

Table (3) Temporal dynamics of serum MDA ng/ml levels following exposure to Gram-resistant & responsive bacteria and lipopolysaccharide (LPS).

Groups	Time (days) after dosing (mean $\pm$ SD)			P value	LSD
	Two	Four	Six		
G1 Control	54.40 $\pm$ 6.7 ^{ab} A	87.93 $\pm$ 8.19 ^{bc} B	71.17 $\pm$ 20.75 ^a AB	0.0201*	21.51
G2 Staphylococcus	45.37 $\pm$ 11.16 ^a B	72.46 $\pm$ 26.05 ^{abc} BC	91.16 $\pm$ 8.93 ^a C	0.0135*	27.45
G3 Streptococcus	40.86 $\pm$ 1.48 ^a A	51.82 $\pm$ 11.1 ^a A	90.16 $\pm$ 9.15 ^a B	0.000031**	13.27
G4 Salmonella	73.10 $\pm$ 16.38 ^{ab} A	95.02 $\pm$ 2.97 ^c A	61.49 $\pm$ 23.82 ^a A	0.0537	NS
G5 Klebsiella	81.48 $\pm$ 2.23 ^b A	60.85 $\pm$ 21.59 ^a A	67.30 $\pm$ 32.01 ^a A	0.4425	NS
G6 LPS(2.5 μ g/Kg)	145.9 $\pm$ 57.3 ^c C	88.58 $\pm$ 1.48 ^{bc} BC	65.36 $\pm$ 35.73 ^a A	0.04367*	62.40
G7 LPS(5 μ g/Kg)	44.73 $\pm$ 1.48 ^a A	69.23 $\pm$ 14.89 ^{ab} A	65.36 $\pm$ 29.78 ^a A	0.2087	NS
P value	0.0000234**	0.00419*	0.443902		
LSD	33.96	22.02	NS		

NS means no significant differences * means significant differences ** means high significant differences

The similarity of the lowercase letters vertically indicates that there are no significant differences between the seven dosing groups.

Different lowercase letters arranged vertically indicate significant differences between the seven dosing groups.

The horizontally identical capital letters indicate that there are no significant differences between the three groups according to time.

Different horizontal capital letters indicate significant differences between the three groups according to time.

Discussion:

The results show an early increase in T-SOD activity on day 2 in both groups, particularly the LPS and *Salmonella* groups, a rapid compensatory response to neutralize free radicals generated following immune stimulation. This pattern is consistent with the known activation of TLR4 receptors, which leads to the production of reactive oxygen species (ROS) as part of the early inflammatory response. Phagocytic cells produce vast quantity of superoxide anions through NADPH oxidase upon activation, which in turn stimulates antioxidant systems as a protective mechanism to maintain oxidative homeostasis [26]. The increase in T-SOD activity persists until day 4, especially in the LPS groups, indicating that the antioxidant response may continue as long as ROS production continues. Studies have shown that sustained activation of antioxidant defense systems



over extended periods is caused by chronic inflammation, which is associated with the continuous production of free radicals [27]. On day six, no significant differences were observed, indicating that T-SOD is a sensitive indicator of the link between inflammation and oxidative stress, and supporting findings that connect biochemical changes with the activation of innate immunity [27,28].

Regarding GSH-Px activity, an early surge in enzyme activity was observed, leading to an increase in ROS and a rapid compensatory response aimed at removing hydrogen peroxide (H_2O_2). Peroxides are thus reduced and their intracellular accumulation is prevented by the glutathione system, which represents the primary cellular defense for maintaining oxidative balance [29,31]. Studies have shown that the reason for cellular adaptation or the gradual consumption of components of the antioxidant system is the increased dynamic activity of GSH-Px in the early stages as a compensatory mechanism, which then tends to stabilize or decrease with continued inflammatory stimulation [30]. In the absence of significant differences, the response would transition to a more stable regulatory phase. Glutathione (GSH) availability has been shown to enhance GSH-Px efficacy, and the ability of cells to regulate ROS levels is also dependent on it [29,31].

Membrane lipid damage is part of the innate immune response, resulting from the stimulation of immune cells. This leads to the release of free radicals due to elevated MDA levels, which represents a clear pattern of oxidative stress [32]. The sustained elevation of MDA levels on day four causes continuous inflammatory stimulation and, consequently, ongoing oxidative stress. Studies show that oxidative stress depends on the intensity and duration of inflammatory stimulation. Increased accumulation of lipid peroxide products indicates persistent inflammation resulting from inadequate antioxidant systems to neutralize them [33]. To reduce lipid peroxides and restore cellular homeostasis, antioxidants such as SOD and GSH-Px work synergistically [29]. Therefore, the activation of innate immunity and the increase in ROS production leads to oxidative damage before antioxidants intervene. From this, we conclude that MDA is a sensitive and reliable indicator for assessing oxidative damage resulting from inflammation [34].



Conclusion:

The results show that activation of innate immunity leads to a clear stimulation of oxidative stress, represented by T-SOD, GSH-Px, and MDA. The early increase in antioxidant activity (T-SOD and GSH-Px) represents a rapid counteracting response to the increase in reactive oxygen species (ROS) due to immune cell activation. The concurrent rise in MDA levels indicates oxidative damage due to lipid peroxidation. The time patterns suggest that the oxidative response occurs first in the early acute phase, characterized by a marked increase in ROS and antioxidant activation. In the second phase, the interplay between oxidative synthesis and antioxidant defense is close. In the third phase, where inflammatory stimulation persists, the response tends toward either relative stability or partial depletion of the defense capacity.

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