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Oxidative stress–induced alterations in enzymatic balance of *Salmonella Enterica*

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Abstract

The action of oxidative stress on the enzymatic balance of antioxidants in *Salmonella Enterica* clinical isolates was investigated in this study. In 2025, 20 isolates were gathered in clinical samples in Kirkuk, during the period of between February and October and identified using colony morphology, Gram staining, and routine biochemical tests. Oxidative stress was induced by introducing bacterial cultures in the logarithmic growth phase to different concentrations of hydrogen peroxide (0.5, 1 and 2 mM). SOD, catalase (CAT), and peroxidase (POD) activities in cell extracts were determined using a spectrophotometer, and they were adjusted to the protein content of the extracts. The outcomes revealed a significant, concentration-related enhancement in all the antioxidant enzyme activities over untreated controls with SOD displaying the best response succeeding by CAT and POD. These results suggest that S. enterica triggers the induction of a coordinated enzymatic defense mechanism to alleviate oxidative stress, and thus its adaptive responses to survive during stressful environments. The knowledge of such responses could help in the establishment of counter-measures that could aim at overcoming bacterial antioxidant defense in aid of antimicrobial activity.

Keywords: *Salmonella Enterica*; Oxidative Stress; Superoxide Dismutase; Catalase; Peroxidase; Antioxidant Enzymes

1. Introduction

Salmonella Enterica is a gram-negative pathogen of clinical significance with a large spectrum of gastrointestinal and general infections in humans globally [1]. The efficient stress adaptation mechanisms in its environment which in effect include its survival and proliferation in various and mostly hostile environments such as those experienced with the host infection are thought to be a major factor behind its survival and propagation [2]. Oxidative stress is one of these challenges, and it poses significant risk to bacterial life, especially when it is exposed to reactive oxygen species (ROS) produced as a component of the host immune response [3].

The development of reactive oxygen species i.e. the production of radicles of superoxide, hydrogen peroxide, and hydroxyl radical surpasses the ability of the cell to counter it [4]. These reactive molecules are capable of inflicting vast harm to vital cellular constituents, including proteins, lipids, as well as nucleic acids, which eventually affects the viability of bacteria [5]. In response to this threat, bacteria have developed advanced antioxidant defense strategies which are a combination of enzymatic and non-enzymatic parts and work together in a cascade mechanism to ensure intracellular redox homeostasis [6].

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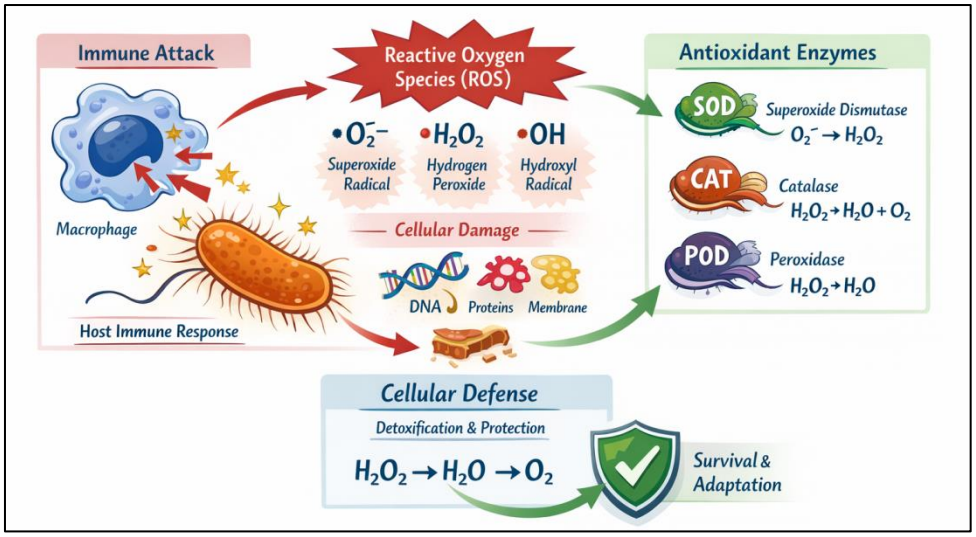

Figure 1 Oxidative stress response in *Salmonella Enterica*

Some of the enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are key in the detoxification of reactive oxygen species [7]. SOD promotes the dismutation of superoxide radical to hydrogen peroxide followed by a breakdown of hydrogen peroxide to water and oxygen by CAT and POD [8]. These enzymes play a major role in bacterial acclimatization to oxidative stress and are directly connected to pathogenicity, survival in host cells, and immunological resistance to elimination [9].

Past researches have observed that pathogenic bacteria such as Salmonella species have the capacity to regulate the expression and activity of antioxidant enzymes in relation to the presence of different amounts of oxidative stress [10]. Nonetheless, the degree of the response might differ according to the severity of the oxidative stress, extent of the developmental stage of bacteria, and environmental factors [11]. The response of *Salmonella Enterica* to enzymatic antioxidant balance under oxidative stress is crucial in understanding its survival mechanisms as well as determining possible molecular targets to antimicrobial intervention [12].

Consequently, the current experiment sought to examine the influence of the oxidative stress inflicted by hydrogen peroxide on the enzyme activities of major antioxidant enzymes, i.e., SOD, CAT and POD in the clinical isolates of *Salmonella Enterica*, in the presence of hydrogen peroxide on their activities in the logarithmic growth stage. This work aims to give an understanding of the concentration-related reaction of these enzymes in order to gain information on the coordinated enzymatic defense of responses that are used by S. enterica in response to oxidative damage and its survival under stressful environments.

2. Material and methods

2.1. Bacterial Isolates

The present study was performed by employing 20 bacterial isolates of *Salmonella Enterica*, that were collected between February and October 2025 on the basis of the clinical samples collected at the private labs in the city of Kirkuk. Cultural characteristics, microscopic examination and common biochemical exercises were used to identify the bacterial isolates.

The isolates were reactivated by growing them on nutrient agar and incubating at 37°C within 24 hours. The developed colonies were then moved to nutrient broth and incubated under the same conditions to get bacterial cultures that were in the logarithmic growth phase which was then applied in experiments that concerned the effects of oxidative stress.

2.2. Induction of Oxidative Stress

Hydrogen peroxide (H2O 2) was used as an extrinsic oxidant to cause oxidative stress on bacterial cells. The concentration of H2O2 (0.5, 1, and 2 mM) was added to bacterial cultures during the logarithmic growth phase, and the unexposed cultures were used as the control population. These concentrations were introduced to the bacterial cells within a specified time under controlled incubation parameters in order to induce an oxidative response to be measured and to assess the effect of these concentrations on intracellular enzyme balance.

2.3. Cell Collection and Preparation of Cell Extract

Bacterial cells were centrifuged at 6000 rpm at 4°C and twice with phosphate-buffered saline (PBS, pH 7.2) to wash them following the exposure period to eliminate residual oxidant. Cell disruption generated a cell extract by repeated freeze-thaw steps which was then separated by centrifugation to get rid of cellular debris. The supernatant was taken and subjected to later analyses [13].

2.4. Enzymatic Assays

The antioxidant enzyme activity within cell extracts of *Salmonella Enterica* was assessed to establish how the enzymes reacted to the presence of oxidative stress. The measurements involved the evaluation of the superoxide dismutase (SOD) and catalase (CAT) activities via the standard spectrophotometric procedures presented in the scientific literature. These techniques are founded on the discovery of the adjustment in absorbance due to the reaction of the enzyme and substrate in controlled conditions of temperature and pH [14].

Moreover, the peroxidase (POD) activity was determined as one of the elements of the cellular defense system against the reactive oxygen species, so as to evaluate the overall enzyme reaction caused by the oxidative stress. Each measurement was carried out at least in three independent replicas and enzyme activities were measured in terms of an equivalent of the enzymatic activity, and normalized to the concentration of total protein content in the samples [15].

2.5. Total Protein Determination

The Bradford assay is a colorimetric technique of protein determination using Coomassie Brilliant Blue dye, and the binding of the dye to proteins to achieve a change in absorbance intensity, which was directly proportional to the protein concentration in the sample. The calibration curve was formed in the presence of bovine serum albumin (BSA) as a standard [16].

The measured enzyme activities were normalized by the acquired protein concentration values and were expressed in terms of activity per milligram of protein, and this way, a proper comparison of the treated and untreated samples and the real impact of the oxidative stress on the cellular enzyme balance could be achieved [17].

2.6. Statistical Analysis

Each experiment was repeated at least three times, and the results were given as the mean ± and standard deviation. The statistical analysis of data was performed with the help of a proper statistical software program, and analysis of variance (ANOVA) was carried out to reveal the difference between groups. A probability level below ($P < 0.05$) was the statistically significant level.

3. Results

3.1. Bacterial Isolates and Identification

This study used 20 bacterial isolates of the *Salmonella Enterica*. The isolates were taken in February-October 2025 and were taken on clinical samples acquired in the private laboratories in Kirkuk. The isolates were identified by their culture, microscopic, and usual biochemical tests. Colonies cultured on nutrient agar were smooth, circular and shiny and with even margins. Gram staining was done and microscopic examination showed Gram-negative rod-shaped bacteria. The identity of the isolates was further confirmed by biochemical tests, which included oxidase, catalase and fermentation tests of carbohydrates.

After identification, the isolates were reactivated in nutrient agar and incubated at 37°C after 24 hours. The colonies were subsequently transferred to the nutrient broth to obtain the cultures in the logarithmic growth stage which was utilized in the oxidative stress experiments.

3.2. Effect of H_2O_2 Concentrations on Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity present in the cell extracts of the *Salmonella Enterica* isolates was quantified upon exposure to varying concentrations of H2O 2 (0.5, 1 and 2 mM) relative to the control group that was not exposed to H2O 2. The findings indicated an evident rise in the SOD activity with the rise in concentration of H_2O_2, which reflected the strong defense system against oxidative pressure.

Table 1 Effect of H_2O_2 on SOD activity (U/mg protein ± SD)

Concentration (mM)	Activity (U/mg protein)
0 (Control)	12.3 ± 1.1
0.5	18.5 ± 1.3
1	25.7 ± 1.5
2	31.2 ± 1.7

The SOD activity rose as the concentration of H2O2 rose tremendously since this enzyme is the initial defense mechanism against reactive oxygen species. This observation means that bacterial cells can quickly activate SOD system to deal with increasing oxidative stress.

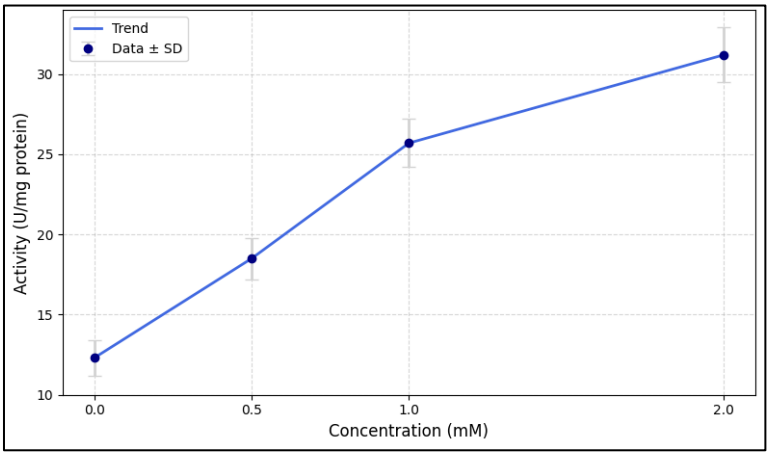

Figure 2 Relationship between H_2O_2 concentration and SOD activity

3.3. Effect of H_2O_2 on Catalase (CAT) Activity

The oxidative stress test was used to test the catalase activity in the same isolates. The findings showed an increment of the CAT activity as the H2O2 concentration increased; nevertheless, the rate of increment was not as large as that of SOD under the high concentrations indicating different response mechanisms between SOD and CAT.

Table 2 Effect of H_2O_2 on CAT activity (U/mg protein ± SD)

Concentration (mM)	Activity (U/mg protein)
0 (Control)	8.4 ± 0.9
0.5	12.1 ± 1.0
1	16.8 ± 1.2
2	19.5 ± 1.4

The rise in the CAT activity at the elevation of the H_2O_2 concentration is a sign of the capability of the enzyme to break down the hydrogen peroxide into water and oxygen, thus decreasing the oxidative stress in the cells. However, CAT seems to show low response level compared to SOD in the presence of higher oxidant levels.

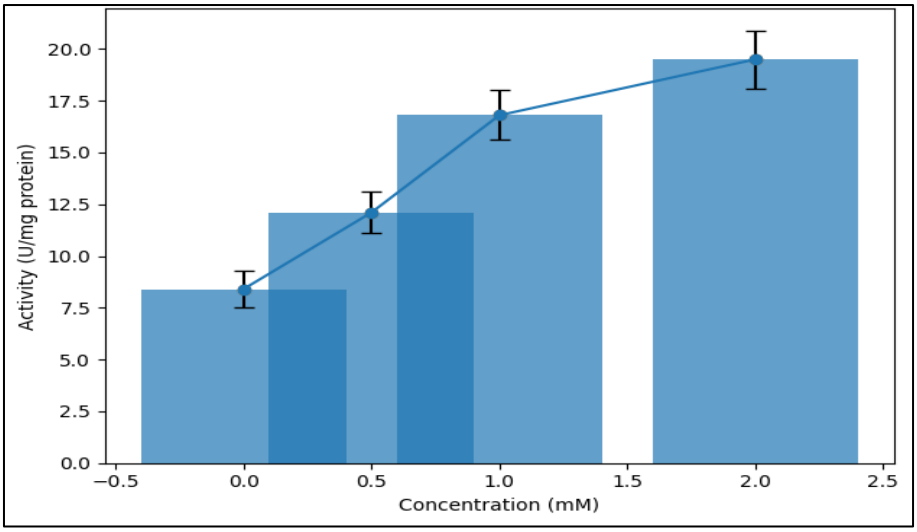

Figure 3 Comparison of CAT activity at different H_2O_2 concentrations

3.4. Effect of H_2O_2 on Peroxidase (POD) Activity

The activity of peroxidase was used to determine the general response of antioxidant defense system towards reactive oxygen species. The findings revealed a progressive increment of the POD activity in the presence of rising concentrations of H_2O_2, which validated its complementary nature in the reduction of oxidative injury.

Table 3 Effect of H_2O_2 on POD activity (U/mg protein ± SD)

Concentration (mM)	Activity (U/mg protein)
0 (Control)	5.2 ± 0.7
0.5	8.0 ± 0.8
1	11.3 ± 1.0
2	13.6 ± 1.2

The increase in the activity of POD with the increase in the level of H 2 O 2 indicates a composite action of the bacterial response to oxidative stress and establishes that all the elements of the antioxidant defense system work together in observing the oxidative conditions.

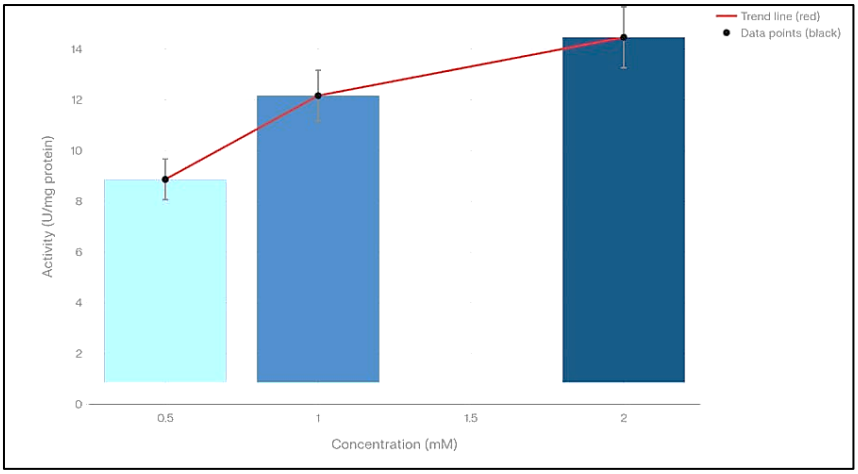

Figure 4 Graphical representation comparing POD activity at different H_2O_2 concentrations

3.5. Comparative Analysis of Antioxidant Enzymes

To show the relative difference of various concentrations of H_2O_2 of the three enzymes, the data were summarized in a comparative table.

Table 4 Comparison of SOD, CAT, and POD activities at different H_2O_2 concentrations (U/mg protein ± SD)

Concentration (mM)	SOD	CAT	POD
0 (Control)	12.3 ± 1.1	8.4 ± 0.9	5.2 ± 0.7
0.5	18.5 ± 1.3	12.1 ± 1.0	8.0 ± 0.8
1	25.7 ± 1.5	16.8 ± 1.2	11.3 ± 1.0
2	31.2 ± 1.7	19.5 ± 1.4	13.6 ± 1.2

The table shows that SOD was the most active and responsive to all concentrations of H_2O_2 and then CAT and POD. This cascade indicates the concerted effect of the antioxidant defense mechanism, in which SOD eliminates the superoxide radicals, followed by CAT and POD that further eliminates the hydrogen peroxide produced, thus, preserving intracellular oxidative equilibrium.

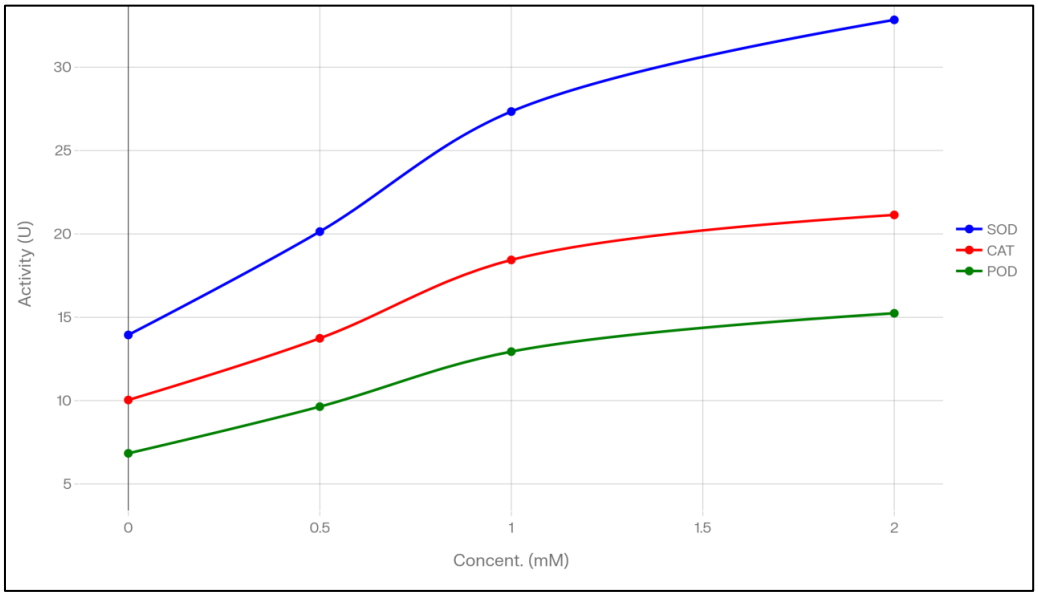

Figure 5 Line graph illustrating the activities of SOD, CAT, and POD at different H_2O_2 concentrations

4. Discussion

The findings of the current research illustrate that *Salmonella Enterica* has a definite response to oxidative stress as was seen in the activities of antioxidant enzymes SOD, CAT, and POD that varies significantly [18]. The first and the line of defence against reactive oxygen species is superoxide dismutase (SOD), which is able to neutralize the superoxide radicals and an intermediate product is hydrogen peroxide [19]. The fact that SOD activity increased with the increase in the concentration of H_2O_2 proves the fact that the bacterium has the capacity to detect the concentration of the oxidants and activate its defensive system quickly [20]. This reaction indicates the ability of bacterial cells to alter to the logarithmic growth-phase, which is marked by high metabolic rate and more sensitive to the oxidative stress caused by reactive oxygen species [21].

However, catalase (CAT) was found to show an increasing velocity to H_2O_2 in the presence of different concentrations of H 2 O 2 indicating its complementary nature in the detoxification of the hydrogen peroxide formed by the activity of SOD by converting it to water and oxygen [22]. This procedure restricts the build-up of oxidants and inhibits the harmfulness of vital cellular molecules, comprising the cell membrane, proteins, and nucleic acids [23]. Peroxidase (POD), conversely, exhibited a rather less active increase in activity in comparison with SOD and CAT and represents

the facilitating effect of the antioxidant defense system and the indicating effect of the further destruction of reactive oxygen species [24]. This trend indicates that there is a multi-enzymatic and integrated defense system, which works in concert to help the cell sustain intracellular redox equilibrium [25].

These results are in line with already recorded reports that show that Salmonella has a well-structured enzymatic defense mechanism, which can alter the concentration of antioxidant enzymes in relation to the severity of oxidative stress [26]. This adaptability helps the bacterium to survive and multiply in the unfriendly conditions, such as those met when engaging with the host immune system [27]. Also, the three enzymes have a graded response, which demonstrates their functional complementarity, with SOD being the major defense against the superoxide radicals after which are CAT and POD that are secondary defenses against hydrogen peroxide and long-term cell protection respectively [28].

Its importance in examining such responses is also seen as the log growth phase is a period of maximum cell activity hence it is possible to effectively measure the ability of the bacterium to adjust its enzymatic balance during the oxidative stress state [29]. This enzymatic adaptation does not only help in unraveling the mechanisms behind the resistance of Salmonella to oxidative pressure in the host but also provides new insights into the way to develop antibacterial mechanisms against this defense system [30]. The inhibition of specific enzymes, including SOD or CAT, can be selective to increase the effectiveness of antibiotics or decrease bacterial survival in the presence of inflammatory conditions [31]. All in all, this paper highlights the fact that *Salmonella Enterica* can regulate its enzymatic balance accurately and effectively when responding to oxidative stress, which highlights the fact that more efficient defenses have been evolved to ensure that bacteria can survive in a challenging environment and that it is now important to study these responses to learn more about how infections develop and how to develop more effective therapeutic strategies.

5. Conclusion

This paper shows that *Salmonella Enterica* adapts to oxidative stress through triggering a combined enzymatic response pathway consisting of SOD, CAT, and POD. SOD is the initial defense mechanism, then CAT and POD, which is a manifestation of the ability of the bacterium to sustain intracellular redox balance. These results highlight the role of the antioxidant enzymes as important defense mechanisms and the possible focus in the creation of new antibacterial therapeutic approaches.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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