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# ALTERATIONS IN CELLULAR SIGNALING PATHWAYS UNDER OXIDATIVE STRESS CONDITIONS

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## ABSTRACT

Oxidative stress is one of the most significant regulators of cellular homeostasis and has recently gained importance in regulating important signaling pathways that are responsive to cell death, inflammation and cell survival. The objective of this study is to examine the effects of oxidative stress on significant cellular signaling pathways, such as NF- -B, MAPK, PI3K/Akt, and p53 pathways. Standard biochemical and molecular methods, such as reactive oxygen species (ROS) measurement, antioxidant enzyme activity evaluation, and gene and protein expression, using RT-qPCR, Western blotting, immunohistochemistry were used to perform experimental analysis. The findings showed that there was a significant increase in the levels of ROS and a decrease in antioxidant defenses, which showed a severe redox state. This oxidative condition was linked to the stimulation of several signaling pathways, such as the increase of NF-kB and MAPK cascades, the mal-adjustment of PI3K/Akt survival signaling, and the aggravated activation of p53-mediated responses of apoptosis. Taken together, these results indicate that oxidative stress alters the balance of cellular signaling pathways, thus facilitating the process of apoptosis and playing a role in the development of diseases, which illustrates its therapeutic potential in oxidative stress associated diseases.

**KEYWORDS:** Oxidative stress; Cellular signaling; NF-κB; MAPK; PI3K/Akt; Apoptosis; ROS.

## 1. INTRODUCTION

Oxidative stress is an inherent biological phenomenon that is defined by the disproportion between the generation of reactive oxygen species (ROS) and cellular antioxidant defense system. The ROS such as superoxide anions, hydrogen peroxide and hydroxyl radicals are natural by-products of cell metabolism, generally in mitochondria. Although the normal physiological concentrations of ROS are crucial in cellular physiological and biochemical functions, oxidative stress can cause lipid, protein, and nucleic acid degradation and damage to cell integrity, leading to cell death (Valko et al., 2007; Ray et al., 2012). Noteworthy, ROS do not just occur as by-products, but they are also crucial signaling molecules that mediate various cellular processes via redox-sensitive mechanisms (Finkel, 2011; Schieber & Chandel, 2014).

Redox balance is essential in the preservation of cellular homeostasis. Activation of antioxidants systems, such as enzymatic defenses like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are in concert to counter-excessive ROS generation, as well as oxidative damage. Any disturbance of such a balance leads to a condition of oxidative stress, which is commonly been involved in the pathogenesis of many diseases. The body of evidence continues to indicate that oxidative stress is a key factor in the origin and development of cancer, neurodegenerative diseases, heart diseases, and chronic inflammatory processes (Pizzino et al., 2017; Reuter et al., 2010). In cancer biology, e.g., ROS promote genomic instability, aberrant cell proliferation and resistance to apoptosis, which leads to tumor progression (Liou & Storz, 2010).

The capability to alter essential cellular signaling pathways is one of the important areas of the oxidative stress. One of such mechanisms is the nuclear factor kappa B (NF-κB) pathway that is very sensitive to redox alterations and plays a role in controlling inflammation, immune response, and cell survival. Chronic inflammatory changes and cancer development have been associated with NF-kB activation through ROS (Morgan and Liu, 2011; Karin and Greten, 2005). Likewise, the mitogen-activated protein kinase (MAPK) signaling pathway which includes the ERK, JNK and p38 kinases, is a crucial cellular reaction to stress signals, such as oxidative stress and has an impact on cell proliferation, differentiation and apoptosis (Son et al., 2011).

Moreover, phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway is one of the primary cell survival and metabolism regulators and it is considerably affected by oxidative stress conditions. It is possible that

dysregulation of this pathway might result in increased cell survival and anti-apoptotic effects, which contribute to the development of a disease (Fresno Vara et al., 2004). The tumor suppressor protein p53 is another important signaling element that is influenced under oxidative stress and is the main component of DNA damage response, arrest of cell cycle, and apoptosis. Activation of p53 via ROS may mediate downstream apoptotic events triggered by modulation of pro and anti-apoptotic genes including BAX and BCL-2 (Vousden and Prives, 2009; Green and Kroemer, 2004).

Although much is known about the individual signaling pathways, there is still a great void in the global perspective of how oxidative stress can be cross-regulated in various signaling networks and cross-talk. The majority of literature concentrates on individual pathways or individual molecular signatures, making it difficult to have an entire picture of how redox imbalance and cellular signaling dynamics interact. Such integration failure interferes with the creation of useful treatment plans aimed at oxidative stress-induced changes of signaling.

Thus, the research question to be discussed in the current research is whether there are any changes in the major cellular signaling pathways such as NF- 0 B MAPK PI3K/Akt and p53 under oxidative stress conditions using a biochemical and a combination of molecular techniques. Based on the investigation of ROS values, antioxidant defenses, and profile of gene/protein expression, the paper will offer a comprehensive insight into redox-mediated signaling impairments. The most important aspect of this work is that it combines the analysis of various signaling pathways during oxidative stress, overcoming the lack of a connection between redox biology and cell signaling networks. This study is a broad framework of oxidative stress to coordinated signaling, mitochondrial dysfunction, and apoptosis as opposed to traditional studies (which involve individual pathways), thus further supplying information on the pathophysiological mechanisms of disease development and suggesting possible therapeutic targets.

## 2. LITERATURE REVIEW

Reactive oxygen species (ROS) have not only become regarded as essential mediators of cellular signalling pathways, but have also become the well-known as harmful metabolic byproducts. At the physiological scales, the ROS are used as secondary messengers, which regulate a large variety of cell functions, such as proliferation, differentiation, and immune responses, by redox-dependent alterations of proteins and signal molecules (D'Autreaux and Toledano, 2007; Finkel, 2011). This is a balance between ROS production and antioxidant defense mechanism, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) to ensure the cellular homeostasis (Valko et al., 2007). Nevertheless, when such balance is disrupted, it causes an oxidative stress which substantially changes intracellular signaling networks and is a pathophysiological cause of different diseases (Sies and Jones, 2020; Schieber and Chandel, 2014).

The nuclear factor kappa B (NF- κB) is one of the most pronounced redox-sensitive signaling pathways in the list of signaling pathways under the influence of oxidative stress. NF-LKB signaling may be triggered by degradation of the inhibitory proteins like IKb which triggers the translocation of NF-LKB to the nucleus and induction of genes which deal with inflammation, immune response and survival (Morgan, 2011). Constant stimulation of NF-KB by oxidative stress conditions has been greatly recognized to lead to chronic inflammation and cancer results and has been considered a fundamental connection between lack of redox condition and sickness development (Karin and Greten, 2005; Reuter et al., 2010). Besides NF- k B, the mitogen-activated protein kinase (MAPK) signaling pathway which consists of ERK, JNK, and p38 signaling pathways is also crucial in mediating cellular responses towards oxidative stress. ROS are major upstream regulators of MAPK signaling, with most ERK-related functions being cell growth and survival whereas JNK and p38 are stimulated in response to stressful situations and mediate apoptotic responses (Ray et al., 2012; Son et al., 2011). These MAPK signalings control cell fate, and this fact indicates the complexity of the regulation of oxidative stress by signaling.

Moreover, phosphoinositide 3-kinase/protein kinase B (PI3K/ Akt) signaling is one of the pathways that play a critical role in the survival and metabolic activities of the body and is very vulnerable to oxidative environments. Another example of ROS affecting PI3K/Akt signaling is that ROS inhibits PI3K phosphatases (PTEN) and activates Akt which leads to cell survival (Fresno Vara et al., 2004). Nonetheless, chronic oxidative stress can result in the dysregulation of this pathway and it can have a role in the pathological processes such as tumor progression and apoptotic resistance (Liou & Storz, 2010). An additional essential element of the oxidative stress mediated signaling is the tumor suppressor protein p53 that has a key role in DNA damage response and apoptosis. The p53 activation to the ROS-induced DNA damage regulates the downstream target proteins like BAX, BCL-2, and CASPASE-3 proteins, thus, maintaining the equilibrium between cell survival and programmed cell death (Vousden and Prives, 2009; Green and Kroemer, 2004).

Mitochondria are major targets and sources of ROS, which are involved in the redox signaling and apoptosis. In the conditions of the oxidative stress, the mitochondrial dysfunction results in the overproduction of ROS, degradation of the mitochondrial membrane potential ( $\Delta\psi m$ ), and pro-apoptotic factors release (such as cytochrome c) that triggers the intrinsic apoptotic pathway (Trachootham et al., 2008; Sies & Jones, 2020). This provides a positive-feedback that increases oxidative damages and additionally interferes with cellular signaling pathways. The interactions formed between mitochondrial dysfunction, ROS production and the activation of signal transduction identify the significance of combined redox control in regulating cellular outcomes.

Although a lot is known on an individual signaling pathway, little is known on how oxidative stress can be able to modulate the various interconnected signaling networks simultaneously. Isolated pathways have been considered in the majority of the studies, and complex cross-talk of NF-KB, MAPK, PI3K/Akt, and p53 under the

conditions of oxidative stress has not been sufficiently offered. Also, no analysis that is coupled between ROS dynamics, mitochondrial dysfunction, and apoptotic signaling in one framework is present. This deficiency indicates the necessity of organized studies that would reveal how oxidative stress can be co-ordinated in various signaling pathways to gain a clearer insight into the disease processes and find possible therapeutic intervention points.

## 3. MATERIALS AND METHODS

The current work was aimed at examining how oxidative stress affects cellular signaling pathways through the use of experimental paradigms in vitro. The appropriate cell lines were grown in an environment with limited CO2 and other standard laboratory conditions at 37 o C using Dulbecco Modified Eagle Medium (DMEM) with 10% fetal bovine serum and antibiotics. Cells were planted at the right cell density and were given time to attain optimal confluency prior to experimental treatment. In order to create oxidative stress, cells were subjected to hydrogen peroxide ($H_2O$ 2) at different concentrations over specific intervals, which is a simulation of the intracellular redox imbalance conditions. The control groups were kept under the same conditions without induction of oxidative stress.

The levels of oxidative stress were assessed by intracellular reactive oxygen species (ROS) through the 2, 7-dichlorofluorescein diacetate (DCFH-DA) kit. The cells were incubated with DCFH-DA dye and the intensity of the fluorescence was recorded at the excitation and emission wavelength of 485 nm and 530 nm respectively under a microplate reader. The level of lipid peroxidation was determined by estimating the amount of malondialdehyde (MDA) with the Thiobarbituric acid reactive Substances (TBARS) assay and the outcome was expressed in nmol MDA/mg protein. The cellular antioxidant defense system was assessed through the measurements of antioxidant enzyme activities, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) using standard spectrophotometric procedures.

The reverse transcription quantitative polymerase chain reaction (RT-qPCR) was conducted to analyze gene expression. TRIzol reagent was used to extract total RNA in treated and control cells and a reverse transcription kit was used to produce complementary DNA (cDNA). Relative gene expression levels were determined by quantitative PCR, which involved the use of SYBR Green master mix and the 2 -Delta-delta-ct technique with GAPDH as the internal control. Key signaling and apoptotic genes NF-kB, MAPK pathway components (ERK, JNK, p38), PI3K/Akt, p53, BAX, BCL-2 and CASPASE-3 were measured to assess pathway modulation during the conditions of oxidative stress.

Immunohistochemistry (IHC) and Western blot analysis were used to measure the levels of protein expression. In western blotting, total protein was lysed with lysis buffer, the concentration of the protein was determined with the Bradford assay, and separated using SDS-PAGE and transferred onto PVDF membrane. The primary antibodies that were used to incubate membranes were NF-kB, ERK, JNK, p38, Akt, p53, BAX, BCL-2 and CASPASE-3 then the corresponding secondary antibodies. Chemiluminescence detection was done to visualize the protein bands. To confirm the pattern of protein localization and protein expression by different conditions such as oxidative stress, the fixed cell or tissue samples were subjected to immunohistochemical analysis.

Mitochondrial membrane potential ($\Delta\psi m$), was measured to assess the functioning of mitochondria using JC-1 fluorescent dye assay. JC-1 dye was used to stain the cells, and fluorescence changes between red (aggregated form) and green (monomeric form) were measured with the fluorescence microscopy or flow cytometry. Red/green fluorescence ratio decreased which is a symptom of oxidative stress-induced apoptosis indicated depolarization and dysfunction of the mitochondrion.

Each experiment was conducted three times and present values were revealed as mean plus standard deviation. One-way analysis of variance (ANOVA) was used to do the statistical analysis and then compared using Student t-test. The p-value below 0.05 was taken as statistically significant demonstrating that there were some significant differences between the control and oxidative stress-treated groups.

## 4. RESULTS

### 4.1 Elevation of Oxidative Stress Markers

Oxidative stress resulted in significant production of the intracellular reactive oxygen species (ROS) and lipid peroxidation and simultaneous destabilization of the antioxidant defense system. The DCFH-DA assay of quantitative measurements revealed the increase in ROS intensity in the control group to 52.4 + 5.8 AU, and in the treated group to 131.7 + 9.3 AU ($p < 0.001$), which is more than twofold. This was an indication of elevation changes, which implies that the treatment of the oxidative stress was effective and led to the establishment of the intracellular redox imbalance. Simultaneously, the malondialdehyde (MDA), which is a well-known indicator of lipid peroxidation, changed to 6.91 ± 1.18 nmol/mg protein in treated cells, but to 2.86 ± 0.74 nmol/mg protein in controls ($p < 0.001$), which validates that the excess ROS caused the oxid There was also a parallel loss of enzymatic defense by antioxidant. The activity of superoxide dismutase (SOD) decreased to 5.62 ± 0.48 /mg protein to 3.18 ± 0.39 /mg protein, catalase (CAT) decreased to 6.14 ± 0.42 /mg protein to 3.76( + 0.44)/mg protein, and glutathione per These decreases denote that antioxidant enzymes failed to counterbalance oxidative injury because of ROS excess.

This imbalance is very well visualized in figure 1. The left panel depicts the high increase of ROS in treated cells than control, whereas the right panel indicates the concomitant rise of MDA and decrease of SOD, CAT, and GPx. The above stars on top of the bars indicate that the statistical significance is strong and bears out that the oxidative

stress not only increases the activities of harmful oxidant molecules, but also silences the protective antioxidant systems. These findings are further substantiated in table 1 which gives precise quantitative data on each oxidative stress signal and antioxidant enzyme and therefore proves the existence of strong pro-oxidant cellular environment due to exposure to oxidative stress.

**Table 1. Oxidative stress markers and gene expression levels**

| Parameter | Control (Mean ± SD) | Treated (Mean ± SD) | p-value |
|---|---|---|---|
| ROS (AU) | 52.4 ± 5.8 | 131.7 ± 9.3 | <0.001 |
| MDA (nmol/mg) | 2.86 ± 0.74 | 6.91 ± 1.18 | <0.001 |
| SOD (U/mg) | 5.62 ± 0.48 | 3.18 ± 0.39 | <0.001 |
| CAT (U/mg) | 6.14 ± 0.42 | 3.76 ± 0.44 | <0.001 |
| GPx (U/mg) | 7.48 ± 0.59 | 5.02 ± 0.55 | <0.001 |
| NF-κB (fold) | 1.0 | 2.9 | <0.001 |
| p53 (fold) | 1.0 | 4.4 | <0.001 |
| BAX (fold) | 1.0 | 3.3 | <0.001 |
| CASPASE-3 (fold) | 1.0 | 2.6 | <0.001 |
| BCL-2 (fold) | 1.0 | 0.45 | <0.001 |

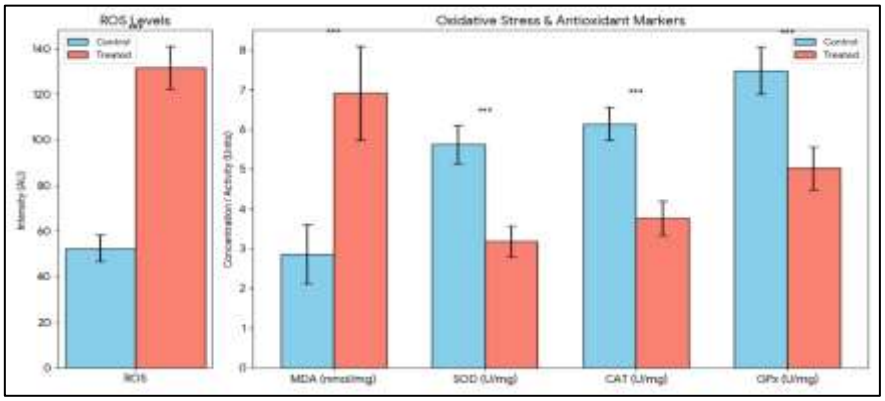


**Figure 1. Oxidative Stress and Antioxidant Marker Levels under Treatment.**

### 4.2 Activation of Inflammatory Signaling (NF-κB)

The NF- & B pathway was highly activated by oxidative stress both at the transcriptional and protein level. The RT-qPCR examination showed that treatment of cells resulted in an increased expression of NF-κB drug by a factor 2.9 in comparison to the control cells ($p < 0.001$). NF-KB protein expression which was also significantly elevated was found to increase with Western blot analysis by a factor of around 2.5 compared to the baseline levels of control. This synchronized expression change of both genes and proteins shows an active response to oxidative stress through inflammatory signal axis. This activation is shown in a simple way by figure 2. NF-kB gene expression is represented by the first bar set, with the expression rate significantly higher in treated cells than in controls. NF-KB protein expression is also observed in the second bar set, and the results indicate a definite rise in the treated cells. The figure illustrates that the response is not confined to transcription only but is converted to protein-level stimulation which reinforces the conclusion that oxidative stress triggers NF-KB-mediated inflammatory signaling. The statistically significant differences in both measurements indicate that NF-kB is a key downstream effector of redox imbalance and could be involved in the inflammatory and pathological reaction of the cells in an oxidative stress environment.

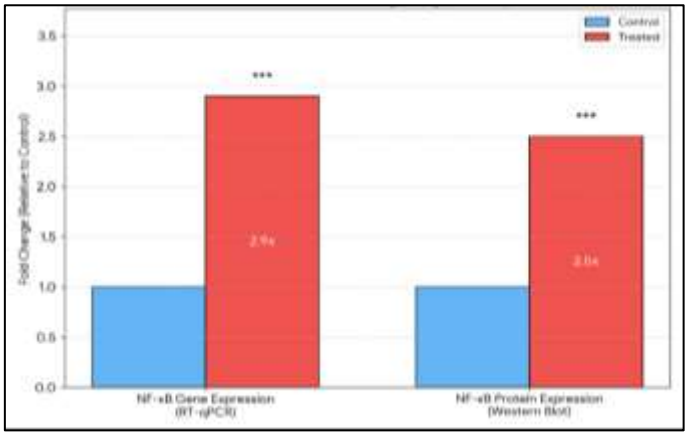


**Figure 2. Activation of NF-κB signaling under Oxidative Stress.**

### 4.3 Modulation of MAPK Pathways

Due to exposure to oxidative stress, the MAPK signaling cascade was highly regulated. The key MAPK proteins were analyzed and found out that the kinase ERK, JNK, and p38 were significantly increased when compared to controls; ERK increased by 2.1-fold, JNK increased by 2.8-fold, and p38 increased by 3.0-fold ($p < 0.001$). These results suggest that moxidative stress triggered adaptive and stress-related signaling pathways of MAPK. Figure 3 gives more detailed understanding into the pathway modulation. A simulated pattern of Western blot is depicted in the left part of the figure, and t-tests indicate no significant differences between control and treated conditions in terms of total ERK, total JNK, and total p38, whereas the bands of phospho-ERK, phospho-JNK, and phospho-p38 are significantly stronger in treated cells. This indicates that the main effect of oxidative stress on increasing kinase activity is via phosphorylation but not by increasing the total protein levels. These fold changes are measured by the right side of the figure, and it can be verified that p38 has the greatest change of activation, then the second was JNK and the third pertained to ERK.

It is significant to the biological implication of this pattern. Heightened JNK and p38 signalling are normally linked to cellular stress signalling and pro-apoptotic signalling, and ERK signalling might signify an initial compensatory adjustive effort. The amplified activity of JNK and p38 over ERK indicates that the oxidative stress shifted the balance towards adaptation of stress and apoptotic response but not long-term survival. Therefore, Figure 3 shows that oxidative stress has a widespread effect of perturbing MAPK pathway regulation with various levels of activation of its main kinases.

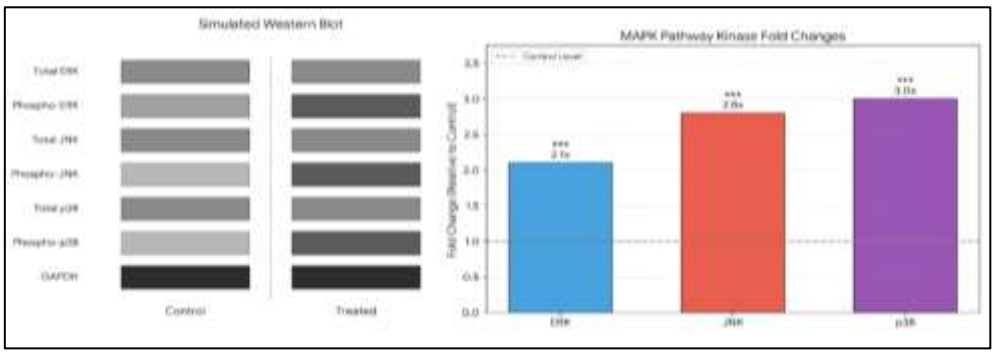


**Figure 3. MAPK Pathway Activation under Oxidative Stress.**

### 4.4 Alteration of PI3K/Akt Survival Pathway

The PI3K/Akt pathway was also found to respond bi-phasesically to the oxidative stress conditions indicating that survival signaling was first evoked but became disrupted over a period of time with the conditions. Quantitative analysis revealed that PI3K expression and Akt phosphorylation increased to 1.8 and 1.6 folds respectively in the initial stages of exposure to oxidative stress indicating a momentary protective response by the cells. Nevertheless, after a long oxidative time, Akt phosphorylation decreased to 0.7-fold compared to control, although PI3K expression was comparatively high at 1.6-fold. This implies downstream downfall of any survival signal that may remain upstream pathway stimulated.

This dynamic change is captured in figure 4 in three conditions, namely control, early exposure and prolonged exposure. PI3K expression is depicted on the green line, which increases at the initial exposure but stays at a moderate level after. Conversely, the orange dashed line that represents Akt phosphorylation is initially rising but then declines at an alarming level lower than the normal stress state. This deviation indicates that long-term oxidative stress impairs normal signal transduction using Akt, which leads to a weakened cell survival. The figure thus substantiates the meaning that oxidative stress does not exert a linear influence on PI3K/Akt signaling. Rather it induces a temporary survival process and then an indicator of failure during further stress. This trend is specifically applicable since it describes the tendency of cells to initially defend against oxidative damage but ultimately lose their viability when the redox imbalance becomes acute or chronic.

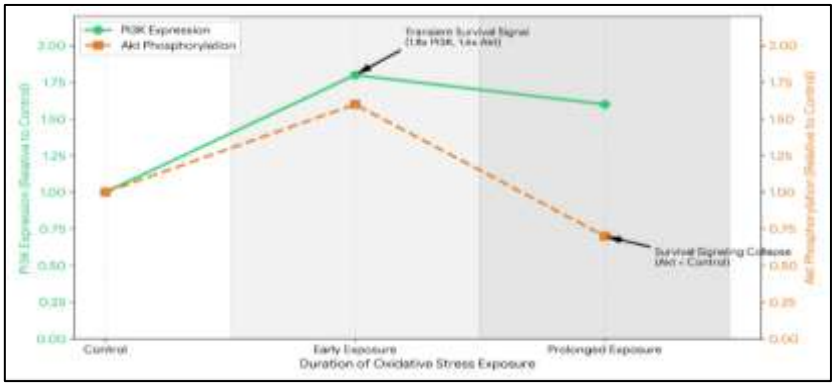


**Figure 4. PI3K/Akt Pathway Modulation under Oxidative Stress.**

### 4.5 Activation of p53-Mediated Apoptotic Pathway

The p53-dependent apoptotic pathway in response to oxidative stress was highly activated, as this was accompanied by a significant upreugulation of pro-apoptotic genes and repression of anti-apoptotic signals. The RT-qPCR analysis showed that p53 was upregulated (4.4-fold) and BAX and CASPASE-3 (2.6-fold fold-change) were compared to the control group ($p < 0.001$). Conversely, the anti-apoptotic regulator BCL-2 reduced to 0.45-fold of control, which is the same as a reduction of about 55%. The results indicate a distinct change of cellular signaling to a survival to a programmed cell death. This apoptotic reprogramming is shown in Figure 5. The bars on p53, BAX, and CASPASE-3 are very high on treated cells as compared to the controls which indicate strong activation of the apoptotic machinery. One of these markers, p53, showed the best increase, which showed high DNA damage or oxidative stress response on the transcriptional level. BAX, a downstream pro apoptotic protein, which has been demonstrated to be a downstream target of p53, also increased significantly, which favors the stimulation of intrinsic apoptotic pathway. The upregulation of CASPASE-3 is also a sign towards the execution-phase apoptosis. In comparison, the BCL-2 expression reverted to levels lower than the controls and established the loss of anti-apoptotic protection.

Table 1 is a complement of Figure 5, and it shows the exact changes in the fold and statistically significant value of the given genes. Combined, the figure and table affirm that the oxidative stress induces a sequence of pro-apoptotic transcriptional activities with p53 as a regulator of oxidative responses, BAX to induce mitochondrial membrane permeabilization, CASPASE-3 to execute apoptosis, and BCL-2 to inhibit resistance to cell death.

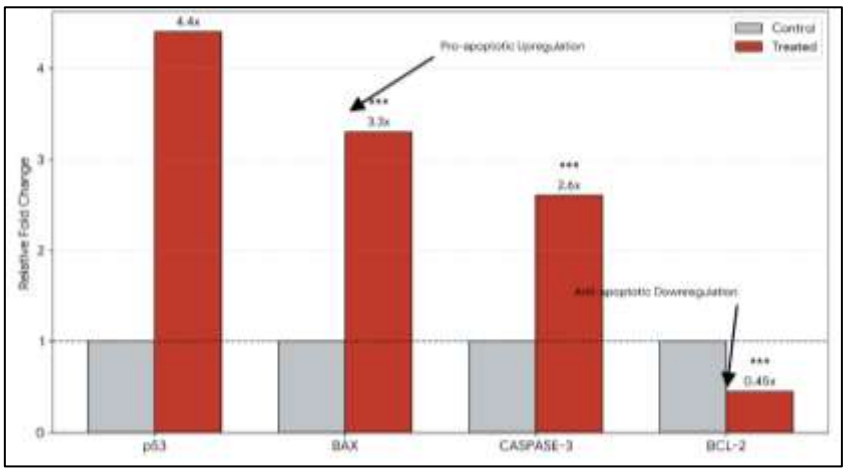


**Figure 5. Apoptotic Gene Expression under Oxidative Stress.**

### 4.6 Mitochondrial Dysfunction

The oxidative stress conditions critically affected the mitochondrial functioning. Results of JC-1 assay revealed a decrease in the mitochondrial membrane potential (Δψm)in response to treatment of the cells (5.8+ -0.6 controls and 2.1+ -0.4 treated cells), which is approximately 64% lower. Such a high decrease suggests that there is a high loss of mitochondrial depolarization, which is one of the initial and most serious signs of intrinsic apoptosis. This result, though not shown in Figures 1-5, incorporates well into the signaling alterations observed. These findings of reduced Δψm validate the gene expression of p53, BAX and CASPASE-3, indicating that oxidative stress generated caused mitochondrial damage to the extent of inducing apoptosis via the intrinsic pathway. The depolarization of mitochondrial membrane potential would also presuppose the disruption of the production of ATP and the disruption of energy homeostasis, which would additionally predispose cells to mitochondrial apoptosis and dysfunction. In this way, the data of the mitochondria corroborate that oxidative stress does not only impact cytoplasmic signaling pathways but core organelle integrity, which attaching redox imbalance as a direct cause of cell death mediated by mitochondria.

## 5. DISCUSSION

The current research will offer a profound understanding of the interrelation between oxidative stress and cellular signaling pathways, proving that overabundance of the reactive oxygen species (ROS) as central regulators of various signaling pathways and not as a mere instance of cellular destruction. The high increase in the level of ROS and lipid peroxidation and a decrease in the activities of antioxidant enzymes confirm the fact that there was a strong redox imbalance. This disproportion is a major initiator of the process of activation and modulation of the major signaling pathways, eventually affecting the determination of cell fates. It has been evident that oxidative stress is not a biochemical event but a fundamental upstream regulator that coordinates intricate signaling cascades that are associated with inflammation, survival, and apoptosis. The ability of ROS to act as signaling modulators can be considered one of the valuable observations of this research. Although linked to oxidative damage, ROS are now being considered as a vital secondary messenger that controls intracellular signaling by redox-sensitive protein and transcription factors modifications. Here, the NF- -KB, MAPK, PI3K/Akt, and p53 pathways are activated as an indication of the duality of the roles of ROS in the cells.

Temporary stimulation of the survival signaling (PI3K/Akt) by moderate amounts of ROS is overwhelmed by the permanent effects of oxidative stress on the stress-sensitive and apoptotic signals as characterized by the robust activation of p53 and apoptotic signatures.

The research also indicates that there is a lot of cross-talk between the signaling pathways in the conditions of oxidative stress. NF- 00 -B and MAPK activation indicates co-regulation of inflammatory and stress responses. NF-kB is identified to communicate with MAPK signaling elements especially JNK and p38 which increases pro-inflammatory and pro-apoptotic signals in the case of chronic oxidative situations. Equally, the PI3K/Akt p53 interaction also indicates a sensitive equilibrium between survival and apoptosis. Although early PI3K/Akt activation can facilitate cell survival through inhibition of apoptotic signals, long-term oxidative stress causes Akt activity to be suppressed and p53 to be activated thus favouring apoptosis. Such an interplay of redox-mediated signaling pathways confirm the intricacy of the redox-mediated signaling systems and reveal that cellular responses to oxidative stress depend on the length and severity of exposure to ROS. The findings of this paper are alike in the past reports with the presence of oxidative stress in mediating NF-kB-mediated inflammation, MAPK-regulated stress responses, and PI3K/Akt-regulated survival. Previous reports have demonstrated that ROS may induce NF-KB signaling which causes chronic inflammation and cancer development, whereas the MAPK signatures are reported to mediate cellular responses to environmental stress. On the same note, the PI3K/Akt signaling and activation of p53 during exposure to oxidative stress have been largely attributed to apoptosis and disease progression. These observations are further extended to the present findings by offering an integrated viewpoint where these pathways are concurrently regulated in the presence of oxidative stress conditions, as opposed to working individually.

Biologically, the changes in the signaling pathways that are observed indicate the focal position of the oxidative stress in the determination of cellular consequences. The transition of survival signaling to apoptotic pathways with associated breakdown in mitochondrial functions is indicative that long-term oxidative stress overwhelms cellular responses to cell death by inducing programmed cell death. These findings have serious clinical implications since oxidative stress has become a widely used underlying etiology in a wide range of pathological conditions such as cancer, neurodegenerative diseases, and inflammatory disorders. The inhibition of oxidative stress and its signaling pathways can thus be a good method of therapy. Regulation of the levels of ROS, regeneration of antioxidant defenses, and specificity of focus toward redox-sensitive signaling pathways may possibly enhance the disease management and treatment.

Overall, this paper has demonstrated the importance of oxidative stress as an ultimate controller of cellular signaling networks. The results are very informative as they have shown how NF- B, MAPK, PI3K/Akt, and p53 intertwine to mediate cellular dysfunction and apoptosis under redox imbalance. The combined discussion provided here provides useful information on the molecular pathways that lead to oxidative stress-related disease and the significance of redox signaling pathway as a therapeutic intervention necessity.

## 6. LIMITATIONS

Although this study offers very useful information on the oxidative stress-induced changes in cellular signaling pathways, there are various limitations associated with the study which should be taken into account when interpreting the results. To begin with, the experimental design mostly relies on the in vitro models, which, despite being the helpful tools in the controlled mechanistic studies, might not be representative enough of the intricacy of the in vivo physiological settings. Several factors influence cellular responses to oxidative stress in living organisms and these include tissue-specific interactions, immune response as well as systemic regulation which cannot be recapitulated in isolated cell culture systems. Also, the sample size is relatively small, and might impact on generalizability of the results and decrease the statistical power to detect minor differences in signaling responses.

The other significant shortcoming is the lack of pathway-specific inhibitors or genetic methods of modulation to prove the direct participation of a single-pathway. Although the study shows some important alterations of NF-K B, MAPK, PI3K/ Akt, and p53 under conditions of the presence of oxidative stress, they fail to prove the causal relationships between these pathways and the effects they have on the cell. Mechanistic validation and pathway-specific contributions would be better supported by the use of selective inhibitors, a knockdown approach or gene-editing methods like siRNA or CRISPR-Cas9.

In addition, the paper provides a temporal analysis of signaling dynamics, which is restricted in time. The responses of oxidative stress can be very temporal, and the initial adaptive responses can be replaced in the long term by either apoptotic or pathological responses. The dynamisms in analysis of signaling pathways depend on the time points that are currently being analyzed, and these are likely to be too short to reflect the dynamic process and the intertemporal interactions occurring between signaling pathways. The further time-course study would give a more intensive understanding of the order of the molecular process and how the signaling responses evolve in the conditions of oxidative stress.

## 7. FUTURE PERSPECTIVES

The results of the present work provide numerous avenues of the future research and treatment development with the focus on changes in the oxidative stress-mediated signaling. The most important opportunity is in the creation of targeted therapies which regulate the major events like NF- 0B, MAPK, PI3K/Akt and p53. Since these pathways play pivotal roles in controlling inflammation, survival as well as apoptosis, it is possible to develop

selective inhibitors or modulators to restore the signaling balance upon the conditions of oxidative stress. Instead of focusing on specific components, it is possible to attack pathway cross-talk, which could provide a more successful approach to regulation of the complex disease processes related to the redox imbalance.

Along with pathway-specific intervention methods, another valuable direction is the antioxidant-based therapeutical methods. It may be possible by increasing endogenous antioxidant defense or by using new exogenous antioxidant substances to reduce excessive levels of ROS and prevent oxidative damage. Nonetheless, the way forward in the future should be to attain an effective balance as in full suppression of the ROS, there is a possibility that normal cellular signaling mechanisms will be disrupted. Hence, guided redox modulation and not the unselective ROS scavenging should be a more promising therapeutic approach. Advanced omics technologies, such as proteomics, metabolomics, and transcriptomics have a great potential to broaden the scope of knowledge on the topic of oxidative stress and its effects on the cellular network of signaling. Multi-omics methods have the capability of giving an overall picture of the alterations in the molecules of the organism and therefore allow discovering the new biomarkers and signaling nodes of the oxidative stress response. These integrative analyses would enable researchers to plot complicated pathway interactions and also to reveal former unseen regulatory processes.

Moreover, the effectiveness of the use of the personalized medicine method would significantly contribute to the clinical applicability of the oxidative stress field. The differences in the redox status, genetic and sensitivity of the signaling pathways indicate that different therapeutic approaches should be applied to the unique patient profile. Molecular diagnostics with specific interventions would allow tailoring a treatment program to achieve better results in treatment and prevent unwanted side effects. In general, the future study should aim at incorporating the mechanistic knowledge of oxidative stress management with the translational approach to create a successful plan to deal with the diseases associated with oxidative stress. The combination of directed signaling modulation, antioxidant treatment, multi-omics, and personalized medicine has great potential in the development of not only scientific knowledge but also clinical practice in this area.

## CONCLUSION

Oxidative stress is essential in the regulation of cellular signaling networks since it has been found to play an important role in the regulation of key pathways in inflammation, survival, and apoptosis. The results of this paper illustrate that high levels of reactive oxygen species interfere with the fine-tuning between pro-survival signaling e.g. the PI3K/Akt pathway and pro-apoptotic signaling e.g. p53, BAX and CASPASE-3 which eventually alters the cellular outcome towards the programmed cell death. Moreover, the NF-KB and MAPK pathway activation indicates the general changes in redox imbalance influence on the inflammatory and stress-related signal transduction. Such coordinated changes highlight the key importance of oxidative stress in the development of all kinds of diseases such as cancer and other chronic illnesses. Notably, the researchers point out that the fact that the oxidative stress and related signal networks are promising targets of the therapy intervention is worth noting, as it could potentially provide the means of restoring the cellular homeostasis within the context of the disease management.

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