RESEARCH PAPER

Heat Shock Proteins in Germinal Cells of Hydatid Cyst Treated with Hydrogen Peroxide (H_2O_2)

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Abstract

Cystic echinococcosis is a prevalent zoonotic illness affecting people and animals. The larval stage Metacestode of the parasite *Echinococcus granulosus* induces the condition. It is a member of the family Taeniidae, which impacts a broad range of domestic and wild animals. The illness of echinococcosis is widespread in both North and South American countries and in the countries of Asia, Australia and Africa, he also pointed out that the illness is endemic in North African countries. Being resilient complicates therapy and encourages recurrence following effective therapies. Heat shock proteins (HSPs) refold denatured proteins, inhibit aggregation, and stabilize protein conformations under stress. HSPs like Hsp60, HSP70 and HSP90 help *E. granulosus* withstand harsh conditions. Hydrogen peroxide (H_2O_2) promotes cellular oxidative stress. Reactive oxygen species (ROS) from H_2O_2 damage macromolecules and disturb *E. granulosus* cellular function. *E. granulosus's* survival tactics include heat shock protein production, resistance to traditional medicine, and the potential role of oxidizing substances like H_2O_2 in treatment effectiveness. Subsequent DNA extraction from cultivated G1 sheep cell lines and Polymerase Chain Reaction PCR technique . This study used specialized primers to target Heat shock protein genes (*hsp*90-5 , *hsp*70-5, *hsp*60). Toxicity assessment of H_2O_2 on cultivated cells of the G1 strain *hsp*90-5. At 200 µM, gene expression was 3.21 times higher than control. At 350 µM, expression increased 3.92 times. *hsp*70-5 increased by 187.56 times at 200 µM compared to the control. Expression increased 238.92 times at 350 µM, showing a substantial response to H_2O_2 induced stress. *hsp*60, compared to the control, expression increased 2.51 times at 200 µM. Expression increased 3.08 times at 350 µM. The study found that H_2O_2 induced oxidative stress significantly increases the expression of *(hsp*90-5, *hsp*70-5, and *hsp60*) . HSPs control oxidative stress and preserve cellular integrity, making them important targets for stress adaptation and resilience research in microbial systems.

Keywords: *Echinococcus granulosus*, HSPs, H_2O_2, Cystic echinococcosis

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Introduction

Cystic echinococcosis is one of the prevalent Zoonotic diseases between humans and animals. The larval stage metacestode of the parasite *Echinococcus granulosus* causes this disease. It belongs to the family Taeniidae, affecting many domestic and wild animals (Hogea *et al.,*2024) . Two kinds of hosts are needed for the parasite's life cycle since domestic animals are intermediate hosts and canines as definitive hosts. However, humans are sometimes intermediate hosts (Khan *et al.,*2023). The illness of echinococcosis is widespread in both North and South American countries and in the countries of Asia, Australia and Africa, he also pointed out that the illness is endemic in North African countries (Deplazes *et al.,*2017). Due to the complexity of the disease and parasite, treating *E. granulosus* (the cause of cystic echinococcosis) is difficult due to the lack of early diagnosis, treatment options, cyst size and location, oxidative stress resistance, lack of vaccines, immune evasion, and drug resistance (Hajjafari *et al.,*2024). Compounding these issues is the remarkable adaptability of *E. granulosus* (Huang *et al.,*2024). The parasite escapes hosting immune defenses and therapeutic medicines by producing heat shock proteins (HSPs) and other survival strategies to survive oxidative stress (He X *et al.,*2023) This surviving ability complicates therapy and increases the chance of recurrence following effective therapies. HSPs are molecular chaperones that refold denatured proteins, suppress aggregation, and stabilize protein conformations under stress (Jeyachandran *et al.,*2023). Under stress, *E.granulosus* produces HSPs like HSP90, HSP70 and HSP60, helping it survive under harsh conditions (Martínez *et al.,*1999). Hydrogen peroxide (H_2O_2) is a potent oxidizing agent commonly used to induce oxidative stress in biological systems (Jomova *et al.,*2024). In *E. granulosus*, H_2O_2 generates reactive oxygen species

(ROS) which damage macromolecules and disturb cellular activities (Avery, 2011). Thus, this study aimed to investigate the cytotoxicity of H_2O_2 against the germinal cells of *E. granulosus* and to investigate the survival strategies of these cells via HSPs synthesis.

Methods

1.1 *E. granulosus* germinal cell culture

The *E. granulosus* germinal cells of the sheep strain (G1) were already prepared by Dr. Sarmad A. M. AL-Asadi and Dr. Ali A. AL-Ali. These cells were routinely cultured at a temperature of 37°C in an atmosphere of 5% (v/v) CO2 in air in a medium consisting of RPMI 1640 culture medium supplemented with 10% (v/v) foetal bovine serum (FBS, Life Technologies), 100 units ml-1 penicillin/streptomycin. The Cells were routinely subcultured after forming a monolayer. This was done by washing twice using 2 ml of phosphate-buffered saline (PBS) and followed by trypsinization via adding 1 ml of trypsin-versine solution for 2 – 5 minutes. The number of viable cells was determined by staining the cells with Trypan blue dye and then counting them using a haemocytometer.

1.2 Effect of H_2O_2 on germinal cell proliferation

The effect of H2O2 on proliferation was investigated. Briefly, 1 x 10^4 cells per well were seeded in 100 µl of an RPMI 1640 culture media into a 96-well plate at 37°C for 24 hours in an atmosphere of 5% (v/v) CO2 in air. After forming a monolayer, the old culture medium was replaced with various hydrogen peroxide concentrations prepared in the serum-free RPMI 1640 medium and added to the appropriate wells of a 96-well plate. The plate was incubated for 72 hours in the same mentioned conditions. There were three replicates for each concentration and the experiment was repeated three times. The cytotoxic effects of H_2O_2 on the geminal cells were estimated using the Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. In brief, After exposure for 72 hours, the old medium was replaced by 90 µl of a fresh serum-free medium and 10 µl of an MTT solution (5mg/ml) prepared in PBS. The plate was incubated at 37°C for 2 – 4 hours. After that, the MTT solution was replaced with 100 µl of DMSO to dissolve the crystal and incubated at 37°C for 20 minutes. The absorbance was measured at 490 nm. The percentage of cell viability and inhibition was calculated from the absorbance of treated and untreated cells. Half of the inhibitory concentration (IC_{50}) was calculated using GraphPad Prism version 10.

Cytotoxicity assay

Received 4× 10^6 cells from the *E. granulosus* cell line of the sheep strain (G1) as a donation from A.M. Dr. Sarmad Awad Mozan and A. Dr. Ali Abdul Latif Abdul Hassan. The maintenance and prolifeatio of cell lines were performed in sterile 25 C^3 culture flasks inside a culture cabinet. The flasks were incubated in a CO_2 incubator with 5% humidity at 37°C. Cell multiplication was achieved through secondary subcultures from the original cultures after the cells formed a monolayer, reaching 80-90%. After pouring the implant media, wash the flasks twice with 2 ml of Phosphate Buffered Saline (PBS). After adding 1 ml of Trypsin-Versene solution, the flasks stay for 2–5 minute to trypsinizaton. The cells are separated from the culture flasks using an inverted light microscope after 5 minutes in a 37°C incubator with 5% humidity. Centrifugation (3 minutes at 1500 RPM) compacts the cells into a pellet. The cells are suspended in serum-containing culture media by adding 10 ml to the initial culture container and transferring half the amount after complete micropipette mixing. This mixture is incubated at CO_2 with 5% humidity at 37°C to promote cell adhesion and monolayer formation (Al-Ali,2023).

1.3 Investigating the effects of hydrogen peroxide on some *HSP* genes

The effects of H_2O_2 on the expression of some *HSP* genes were investigated. Prior to each experiment, different concentrations (0.0, 200, and 350 µM) of H_2O_2 were prepared in the serum-free RPMI 1640 medium and added to the appropriate 25 cm^2 flasks, containing a monolayer of 2 x 10^6 germinal cells. The treated and untread cells were incubated at 37°C in an atmosphere of 5% (v/v) CO2 in air for one hour. After trypsinization, the cells were pelleted by centrifugation at 1,500 g for 1 min and then washed twice in PBS before being used in RNA extraction. There were 3 replicated flasks for each concentration. The experiment was repeated three times.

1.4 RNA extraction

RNA was extracted from 2 x 10^6 cells utilizing the GENEzol™ TriRNA Pure Kit (Geneaid, Taiwan). The extraction was performed according to the manufacturer's instructions. RNA concentration was evaluated using an Implen™ nanophotometer. The RNA was stored at –80°C until used in the cDNA synthesis.

1.5 cDNA synthesis

The first strand cDNA synthesis was done using 1 µg of germinal cell RNA together with AccuPower® RocketScript™ Cycle RT PreMix (Bioneer), oligo20(dt) and random hexamer primers according to the manufacturer's instructions.

1.6 Quantitative real-time polymerase chain reaction

The quantitative real-time polymerase chain reaction (qPCR) technique was used to determine the expression of *HSP*10, *HSP*60, HSP70_5, hsp90_5. Each reaction contained 10 µl of GoTaq® qPCR Master Mix (Promega), 0.1 µM of each primer, 5 µl of 5-fold diluted cDNA in a final volume of 20 µl. the HSP primers were designed in this study using Primer3 software and validated using conventional PCR followed by electrophoresed on 1% (w/v) agarose gel. The primer efficiency was estimated using qPCR and melt curve analysis was also performed . Thermal cycling conditions were a pre-denaturation step at 95ºC for 2 min followed by either 50 cycles of denaturation at 95ºC for 15 s, annealing at 60ºC for 30 s and extension at 72ºC for 30 s. Threshold cycle (CT) value was calculated from the fluorescence curve for each sample. The transcript abundance for the gene of interest was normalized to the transcription abundance of the β- actine gene using the formula $2^{-\Delta\Delta Ct}$.

by using the GoTaq® Kit 2-step RT-qPCR technique, according to the manufacturer's guidelines, Bioneer, Korea. 8 µl of RNA template, 1 µl of Oligo20(dt) primer, 1 µl of Random hexamer, and 10 µl of DEPC-D.W. were taken.

the The inhibitory concentration value was determined for half of the IC50 cells. It was exposed to H_2O_2 in the cell line after conducting treatment experiments with 3-5 repetitions. Subsequently, the inhibition ratios for all concentrations of H_2O_2 were calculated using GraphPad Prism 7 software.

According to the manufacturer's

Results and Discussion

The study examines how H_2O_2 affects germinal cell growth and heat shock protein gene expression. The main focus is on the cytotoxicity of H_2O_2, its inhibitory concentration (IC_{50}), and its effect on the expression of *hsp*90-5, *hsp*70-5and *hsp*60 Furthermore, *hsp*60 encode proteins translated to the extracellular environment, cytoplasm, and mitochondria. The genes had examined how different concentrations of H_2O_2 affect cell growth, cytotoxicity, and HSPs gene expression. Cystic echinococcosis is a prevalent zoonotic disease that affects both humans and animals, it is caused by the larval stage (metacestode) of the parasite *E. granulosus*, this parasite is a member of the Taeniidae family and infects a diverse range of domestic and wild animals (Hogea *et al.,*2024). Echinococcosis is a widespread illness in both North and South America, as well as in Asia, Australia, and Africa. It is also noted to be endemic in North African countries (Deplazes *et al.,*2017). The treatment of the parasite responsible for Cystic echinococcosis is challenging due to various factors such as its complexity, lack of early identification, limited treatment options, the size and location of cysts, resistance to oxidative stress, absence of vaccines, ability to evade the immune system, and resistance to medications. (Hajjafari *et al.,*2024).

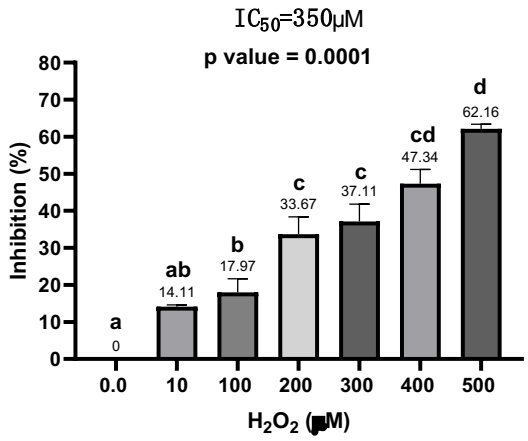
3. 1 The half of Inhibitory concentration IC_{50}

This study investigated the effects of H_2O_2 on cellular proliferation. Different concentrations (0, 10, 100, 200, 300, 400, and 500 µM) of H_2O_2 were used to ascertain the inhibitory concentration for the growth of the cell population (IC_{50}). The designated concentrations were applied to the G1 sheep cell line to evaluate the extent of cell growth inhibition caused by each dose. H_2O_2 is a ROS that induces oxidative stress in cells, leading to different biological effects, the toxic effects of H_2O_2 are buffered by various ROS-scavenging systems (GIORGIO *et al.,2007*).

Two experiments were performed, each using three repetitions to enhance the accuracy of the data. The IC_{50} value indicates the concentration necessary for an inhibit 50% cells growth. It is a crucial metric for assessing the substance's relative toxicity and inhibitory effects on cells. The IC_{50} was determined to be 350 µM. Therefore, this concentration is optimal for demonstrating the impact of H_2O_2 on cell growth in the present study. The control group had no inhibition at 0.0 µM because the percentage was 0%.

At 10 µM, H_2O_2 affects cell growth with a somewhat higher inhibition rate than the control group. Ab represents a concentration of 10 µM, suggesting that its statistically significant variations from 0.0 are negligible, which was 13% inhibition. Compared to the previous two doses, 100 µM of H_2O_2 inhibited cell growth more strongly. The symbol b indicates significant variations from 0 and 10 µM, the rate of inhibited cells were 18% . 200 µM concentration About 38% inhibition. At this concentration, the percentage of inhibition increases significantly, as shown by the symbol "c" compared to lower concentrations (0, 10, and 100 µM). 300 µM concentration About 40% inhibition. This concentration has the same symbol "c" as the 200 µM concentration, suggesting no significant change. Concentration 400 µM, about 55% inhibition. The inhibition ratio increases with concentration, as shown by the symbol "cd." It is not significantly different from 200 and 300 concentrations, but it differs somewhat. 500 µM, concentration about

70% inhibition. The symbol "d" indicates a statistically significant difference from all other concentrations, indicating the highest inhibition in the experiment. H_2O_2 induces cytotoxicity via the generation of oxidative stress, damaging.



(Figure 1): Illustrates the inhibition rates at various concentrations of H_2O_2

The statistics indicate a direct correlation between drug concentration and the inhibition ratio. The inhibition ratio rises with increasing concentration. The IC_{50} value was attained at a concentration of 358 µM, indicating that this concentration results in 50% inhibition. Statistical differences demonstrate that an elevation in concentration results in a significant enhancement in inhibition, peaking at a ratio of 500 µM. The findings demonstrated substantial variations in growth inhibition across different doses. They suggest that little increments in H_2O_2 concentration may elicit large cytotoxic effects. H_2O_2 induces cytotoxicity via the biological macromolecules, including DNA, proteins, and lipids (Schumacker,2006). The gradual suppression of cell growth indicates the buildup of oxidative damage that surpasses the cell's antioxidant defense systems (Andrés *et al.,*2022).

The present study used three genes with distinct cellular loci. The predictive analysis indicated that the *hsp*90-5 encodes secretory proteins, while the *hsp*70-5 encodes cytoplasmic proteins. Moreover, this research revealed that the *hsp*60 encodes mitochondrial proteins. HSPs have complementary functions in preserving cellular integrity and viability under stress conditions (Rojkind *et al.,*2002). Their increase in response to H_2O_2 exposure underscores their protective roles against oxidative stress, facilitating protein stability, cellular repair, and energy generation (Jomova *et al.,*2024).

3-3 Expression of selected HSP genes in Germinal cells

3-3-1 Expression of *hsp*90-5 gene in cells exposed to H_2O_2

The findings of exposing germinal cells to an IC_{50} concentration of 350 µM and a sub-IC_{50} concentration of 200 µM indicated an increase in the gene expression *hsp90-5*, which encodes a secretory protein. The gene expression rate at a concentration of 200 µM of H_2O_2 was more than thrice (3.21) greater than that of the control group. The findings indicated that the transcription rate at an IC_{50} concentration of 350 µM increased more than threefold compared to the control group.

Concentrations (200 and 350 µM) caused significant statistical differences from the control group as indicated in Figure (2).

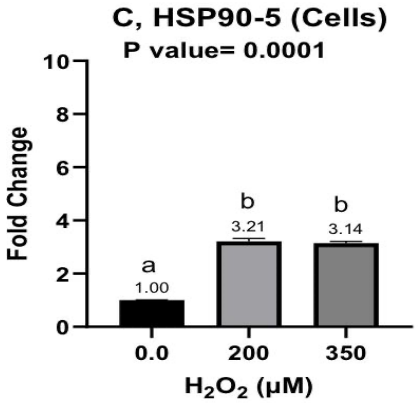


Figure (2) demonstrates gene expression at *hsp90-5* for germinal cells exposed to different concentrations of H_2O_2

The figure depicts the fold change at *hsp 90-5* in germinal cells subjected to 0 µM (Control), 200 µM (sub-IC_{50}), and 350 µM (IC_{50}) concentrations of H_2O_2. In the control group (0 µm), the fold change in gene expression is 1.00, indicating baseline *hsp 90-5* expression without oxidative stress. This helps determine how H_2O_2 affects gene expression. The 200 µM and 350 µM doses exhibit substantial statistical differences from the control group. This demonstrates a consistent and repeatable rise in *hsp90-5* expression throughout the evaluated doses.

The *hsp90-5* produces secretor proteins crucial for mitigating stress-induced damage by promoting proper protein folding and preventing aggregation (Zare *et al*.,2024). The *hsp90-5* is involved in the response to oxidative stress. It suggests that H_2O_2 acts as an oxidative stressor (Mattos *et al*.,2025). Stimulating cells to increase the production of stress-response genes such as *hsp90-5*.

The results unequivocally indicate that oxidative stress caused by H_2O_2 markedly increases the expression of *hsp90-5* in a concentration-dependent manner, exhibiting a pronounced plateau effect at elevated doses. This underscores the gene's essential function in safeguarding cells under oxidative stress.

3.3.2 Expression of *hsp70-5* gene in cells exposed to H_2O_2

The results of exposing germinal cells to an IC_{50} concentration of 350 µM and a sub-threshold concentration of 200 µM showed increased gene expression of cytoplasmic protein HSP70-5. Gene expression was 187 times (187.56) higher than the control group at 200 µM of H_2O_2. At 350 µm IC_{50}, the transcription rate rose 238 times (238.92) compared to the control group. 200 and 350 µM concentrations showed significant differences from the control group. As seen in Figure (3).

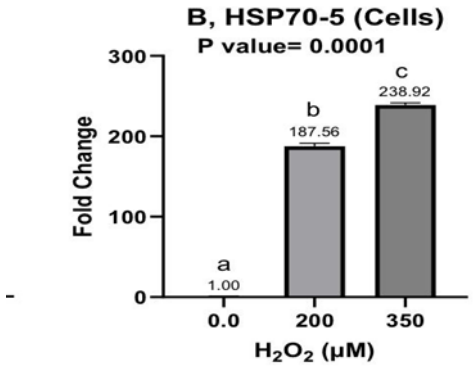


Figure (3) demonstrates gene expression at *hsp70-5* for germinal cells exposed to different concentrations of H_2O_2

The figure illustrates *hsp70-5* expression fold change in germinal cells exposed to H_2O_2 doses. 0 µM (control), 200 µM (sub-IC_{50}), and 350 µM (IC_{50}). Control group (0µM) the fold change in gene expression is 1.00µM, indicating baseline *hsp 70-5* expression without oxidative stress. Under physiological circumstances, gene expression of *hsp 70-5* is normal and unexplored. AT the 200 µM of H_2O_2 was found a significantly increased gene expression, with a fold change of 187.56. This is 187-fold higher than the control group. It indicates that oxidative stress regulates the *hsp70-5*. Biological implication research indicates that sub-inhibition H_2O_2 concentrations provide cytoprotecting by upregulating Hsp70-5, a cytoplasmic chaperone protein essential for protein stability and folding under stress. 350 µM H_2O_2 IC_{50} concentration, the *hsp70-5* expression fold change increases to 238.92 at 350 µM H_2O_2, showing a stronger induction than the control group. The gene expression of the *hsp70-5* is greater at 350 µM than at 200 µM, indicating a dose-dependent response to oxidative stress. The fold change in gene expression at 200 µM and 350 µM doses significantly differs from the control group . This demonstrates the constancy and dependability of oxidative stress-induced upregulation.

Biological Importance: the *hsp70-5* encodes cytoplasmic proteins that function as molecular chaperones, inhibiting protein misfolding and aggregation under cellular stress (Hasan *et al* .,2023). The significant rise in *hsp70-5* expression at both doses indicates that oxidative stress triggers defensive mechanisms to maintain proteotoxicity and reduce damage. The concentration-dependent effect seen at 350 µM suggests that elevated oxidative stress induces a more robust transcriptional response.

The results indicate a robust and concentration-dependent activation of the *hsp70-5* in response to oxidative stress caused by H_2O_2. This underscores the essential function of Hsp70-5 in safeguarding cells from oxidative injury and preserving cytoplasmic protein equilibrium. The statistically significant changes highlight the vulnerability of germinal cells to oxidative stress and the critical role of stress-responsive genes in cellular defense systems.

3.3. Expression of *hsp60* gene in cells exposed to H_2O_2

Exposing germinal cells to an IC_{50} concentration of 350 µM and a sub-IC_{50} concentration of 200 µM indicated an increase in the gene expression of *hsp60*, which encodes the mitochondrial protein HSP60. The gene expression rate at a concentration of 200 µM H_2O_2 was 2.51 times that of the control group. The expression findings at an IC_{50} concentration of 350 µM indicated that the transcription rate increased more than thrice (3.08) compared to the control group. Furthermore, the findings indicated significant statistical differences influenced by

concentrations of 200 and 350 µM compared to the control group. As seen in the figure (4).

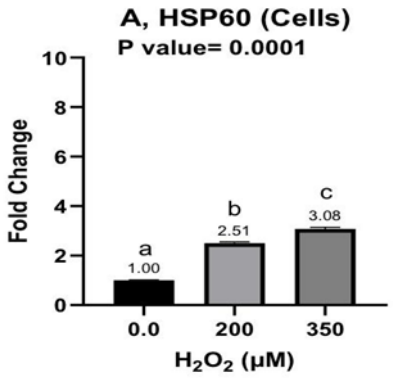


Figure (4) demonstrates gene expression at *hsp60* for germinal cells exposed to different concentrations of H2O2

The figure illustrates the fold change in *hsp60* gene expression in germinal cells exposed to different concentrations of H_2O_2 0 µM (control), 200 µM (sub-IC_{50}), and 350 µM (IC_{50}).

Control Group (0 µM H_2O_2) the fold change in gene expression is 1.00, the baseline reference for Hsp60 expression in normal physiological settings. This indicates the baseline expression level of *hsp60* in the absence of oxidative stress. Sub-IC_{50} Concentration (200 µM H_2O_2) exposure to 200 µM H_2O_2 elevated *hsp60* expression by a factor of 2.51, indicating a 2.5-fold increase relative to the control group. The mild oxidative stress induced by 200 µM H_2O_2 stimulates the mitochondrial stress response, leading to the upregulation of Hsp60, which safeguards mitochondrial proteins and averts malfunction. IC_{50} concentration (350 µM H_2O_2) at 350 µM H_2O_2, the fold change in *hsp60* expression rises to 3.08, indicating a threefold increase relative to the control group. Comparison with 200 µM concentration, the elevation in *hsp60* expression at 350 µM is almost more than at 200 µM, signifying a more robust response to elevated oxidative stress levels.

Biological Significance: this heightened expression indicates that at IC_{50} concentrations, mitochondrial stress is intensified, necessitating an increased transcriptional response to preserve mitochondrial integrity and protein folding (Moniruzzaman *et al*.,2018).

The alterations in hsp60 expression at 200 µM and 350 µM H_2O_2 are statistically significant compared to the control group . This verifies that the observed increase of Hsp60 is not coincidental but a consistent response to heightened oxidative stress.

Hsp60 is a mitochondrial chaperone protein that ensures mitochondrial proteins' correct folding and stability under stress conditions(Mouawad et al.,2023) . Mitochondrial stress response the elevation of Hsp60 expression underscores the activation of defensive mechanisms in mitochondria, which are especially vulnerable to oxidative damage (Zuo *et al*.,2024). This reaction seeks to maintain mitochondrial activity and avert cellular apoptosis under oxidative stress.

The fold change in *hsp60* expression indicates a distinct dose-dependent response, whereby more oxidative stress triggers a more pronounced transcriptional gene activation. Moderate stress (200 µM) A moderate increase in expression indicates a defensive response adequate to manage sub-inhibition oxidative stress. Acute stress (350 µM) elevated *hsp60* expression at IC_{50} indicates a more resilient defensive system against significant mitochondrial injury.

The results highlight the significant upregulation of *hsp60* gene expression in response to oxidative stress induced by H_2O_2 with a dose-dependent increase observed at higher concentrations. This underscores the critical role of Hsp60 in mitigating mitochondrial damage and maintaining protein homeostasis under oxidative stress conditions. The statistically significant differences validate the biological importance of Hsp60 as a key player in the cellular stress response.

Previous studies have shown that relatively low concentrations of H_2O_2 ranging from 10 to 75 µM, can inhibit the proliferation of various human cancer cell lines. For example: The A549 human lung cancer cell line had an IC_{50} value of 10 µM. The HepG2 human hepatocellular cancer cell line had an IC_{50} value of 70 µM. The Calu-6 human lung cancer cell line had an IC_{50} value of 75 µM. The HeLa human cervical cancer cell line had an IC_{50} value of approximately 75 µM. These findings suggest that H_2O_2 can effectively reduce the growth of certain cancer cell lines at relatively low concentrations(; PARK,2013 ; PARK,2014; LUO *et al* .,2016).

These values are comparable to those obtained for the Jurkat T-lymphoma cell line, which has an IC_{50} value of 50 µM. The Jurkat T-lymphoma cell line exhibits a similar response to H_2O_2 as other human cancer cell lines. In contrast, T. thermophila demonstrates significantly higher resistance to H_2O_2 with an IC_{50} value of 60 µM, compared to human cancer cells (Al-Asadi,2018). The values obtained for the Jurkat T-lymphoma cell line, with

an IC_{50} value of 50 µM, are consistent with those observed in other human cancer cell lines in response to H_2O_2. Conversely, T. thermophila exhibits significantly greater resistance to H_2O_2, with an IC_{50} value of 60 µM, compared to human cancer cells(FOURRAT et ak.,2007). The findings of the current study align with previous research conducted on a similar organism, suggesting that insensitivity to H_2O_2 might be a widespread characteristic among all Tetrahymena species and possibly all ciliated protozoans. There is limited research on this phenomenon; however, one example worth noting is that like T. thermophila, the ciliates *Euplotes raikovi* and *E. nobilii* have also shown resistance to H_2O_2. Specifically, *E. raikovi* required 75 µM H_2O_2 for complete inhibition of cell proliferation, while E. nobilii survived even at concentrations as high as 2000 µM H_2O_2(DOBRI *et al*.,2014). The acquisition of thermal resistance is associated with an increased synthesis of proteins known as heat shock proteins (HSPs) (Mc and Finkelstein 1980;Li and Werb 1982). Heat shock genes, responsible for coding HSPs, are transcriptionally activated in response to a range of stressors, such as heat shock (Ashburner and Bonner1979).

Conclusion

The research demonstrates the strong cellular reaction to oxidative stress caused by H_2O_2. The expression of Hsp90-5, Hsp70-5 and Hsp60 is concentration-dependent and the effect of H_2O_2 on these Hsps clarifies the cellular stress response under oxidative conditions. The results demonstrate a concentration-dependent elevation in the expression of three stress-related genes, highlighting the direct effect of oxidative stress caused by H_2O_2. This pattern underscores the gradual activation of protective mechanisms in response to increasing stress levels preserving cellular integrity by ensuring protein stability and functionality. H_2O_2 is commonly recognized as a cytotoxic agent that must be minimized by antioxidant defense enzymes (Singh *et al*.,2024). Exposure to H_2O_2 is a widely used procedure to induce oxidative damage/stress in cellular models (Chen *et al*., 2024).

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Conflict of interest

The authors have no conflict of interest.

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