

## The Effects of Iron ( $Fe^{3+}$ ) on The Expression Levels of Heat Stress Protein Genes in Rat Liver (*Rattus norvegicus*) Tissue

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**ABSTRACT:** Iron, one of the most common metals in the world, is important for organisms. It is known that at high concentrations, it damages especially organs such as liver, pancreas, heart. In our study, the effect of iron ion on the expression of the 70 kDa HSP gene family which small stress proteins was investigated in *Rattus norvegicus*. In this study, iron ion  $Fe^{3+}$  (0,87ppm, 3ppm, 30ppm, 300ppm) was given to 5 different application groups at different concentrations. At the end of this application period, a cDNA library was formed from the liver tissues taken from the living body. Using these libraries, changes in expression levels occurring in the HSP70 (Hspa1a, Hspa4, Hspa5) HSP90 (Hsp90aa1) genes were determined by Real-Time PCR method.

**Key words:** Gene expression, hsp, iron ion, *Rattus norvegicus*, real-time PCR.

## Demir İyonunun ( $Fe^{3+}$ ) Sıçan (*Rattus norvegicus*) Karaciğer Dokusundaki Isı Stres Proteini (Hsp) Genlerinin Ekspresyon Seviyeleri Üzerine Etkisi

**ÖZET:** Dünyada en fazla bulunan metallere birisi olan demir, organizmalar için önemlidir. Yüksek konsantrasyonlarda özellikle karaciğer, pankreas, kalp gibi organlarda hasar oluşturduğu bilinmektedir. Çalışmamızda *Rattus norvegicus*'da demir iyonunun küçük stress proteinleri olan 70 kDa HSP gen ailesinin ekspresyonu üzerine olan etkisi araştırıldı. Bu çalışma kapsamında 5 farklı uygulama grubuna farklı konsantrasyonlarda demir iyonu  $Fe^{3+}$  (0,87ppm, 3ppm, 30ppm, 300 ppm) verildi. Bu uygulama süreci sonunda canlıdan alınan karaciğer dokularından cDNA kütüphanesi oluşturuldu. Yapılan bu kütüphaneler kullanılarak HSP70 (Hspa1a, Hspa4, Hspa5) HSP90 (Hsp90aa1) genlerinde meydana gelen ekspresyon seviyelerindeki değişimler Real-Time PCR metodu ile tespit edildi.

**Anahtar Kelimeler:** Demir iyonu, gen ekspresyonu, hsp, *Rattus norvegicus*, real-time PCR

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## INTRODUCTION

In life, “stress factors” are the internal and external factors that cause physiological balance impairment and the “cell stress response” is the response of the cells to the stress factors (Jolly and Morimoto, 2000). When confronted with stressful situations such as temperature shocks, cells increase the synthesis of a group of proteins which called heat shock or stress proteins. The stress response seen in all living things is shaped by a special gene group that is highly conserved in development (Mezquita et al., 2001). Apart from its hot application, it is known that stress factors such as heavy metals, free oxygen radicals, amino acid analogues stimulate temperature shock protein synthesis in the cell (Lindquist and Craig, 1988). In conditions where there is no stress effect, temperature shock proteins are present in the cells, and these proteins, which are continuously expressed, are called temperature shock cognates and function in the folding, transporting and regulating mechanisms of proteins within the cell (Mathew and Morimoto, 1998; Yeğenoğlu, 2007). During temperature stress, DNA synthesis, transcription, RNA processing, translational events, and stops of cell cycle progression. At the same time, denaturation occurs in proteins, lysosomal degradation events increase, membrane permeability changes, and there are increases in extracellular ion transport. As a result, transcription and translation of heat-shock protein genes begin. In such cases the function of heat-shock proteins, such as Heat Stress Protein 70 (Hsp70), is to prevent the precipitation of denatured proteins by the function of molecular chaperones, to re-fold these proteins properly and to improve the obtained stress tolerance. Oxidative stress is an important signal for the apoptotic process. Changes in the cellular redox state are an effective way to regulate different apoptotic pathways. HSF-1 transgenic mice were found to be more susceptible to oxidative stress (Yan et al., 2002). HSPs act as antioxidants in the protection of the cellular redox state. Oxygenase is an HSP responsible for the production of antioxidants from bilirubin and biliverdin. It is known that Hsp70 enhances peptide complex stability and peptide binding ability under oxidative stress conditions. Redox status in the cell affects Hsp70 synthesis. Thus, reduced GSH levels may lead to direct activation of HSF-1 (Marius and Robert, 1996). On the contrary, strong oxidizing agents inhibit the trimerization of HSF-1 by inhibiting its ability to bind to DNA. As a result, a moderate change in redox homeostasis leads to the activation of HSF-1, while large changes in redox homeostasis inhibit HSF-1 (Sreedhar and Csermely, 2004).

The physiological functions of stress proteins become more important when the cell exposed heat shock. Stress proteins prevent cleavage of oligomeric complexes and opening of polypeptides under heat shrinkage. If re-folding becomes impossible, it accelerates the removal of denatured proteins. On the other hand, the presence of denatured proteins in the cell stimulates the production of stress proteins. Microbial pathogens accelerate the synthesis of stress proteins to protect the host staphylococci produced by host phagocytes. If the intracellular pathogen *Salmonella* is previously treated with hydrogen peroxide, the synthesis of stress proteins increases and this protects it from the effect of hydrogen peroxide in the higher lethal dose. In general, it has been found that mutants producing high-dose stress proteins are resistant to heat and oxidant agents at an advanced level, while mutants with defects in their stress protein genes are highly sensitive to the killing effect of active macrophages (Zuhail, 2009).

## MATERIALS AND METHOD

### Experimental Animal and Experimental Application

Male *Rattus norvegicus* strain Sprague-Dawley rats were obtained at the Experimental Medical Application and Research Center of Atatürk University (Erzurum, Turkey). The rats used in this experiment were housed in a controlled room ( $22 \pm 2$  °C) and humidity (40-60%) and in a room which was regularly lighted for 12 hours in the dark and 12 hours in the desired amount of food and water. The rats were left for at least one week in the period of adaptation with deionized water before starting the experiment. Animals were exposed to a daily mixture of deionized water and metal for 100 days. During the first week to the next week, the ion concentration of the water was increased incrementally until each group was brought to its own nutrient concentration. In this experiment, 5 group of rats were used. The first group used as control was given only deionized water for 12 weeks. Iron concentration ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) was given in the basic dose group (1 group; 3 ppm). The first week of deionized water, at week 2 and after, 0.87 ppm (in concentrations equivalent to the maximum limits determined by WHO) iron ion concentration was applied. The third group was treated with deionized water for the first week, 0.87 ppm for the second week, and 3 ppm for the third week and thereafter. Group 4 was treated with deionized water for 1 week, 0.87 ppm for 2 weeks, 3 ppm for 3 weeks, 30 ppm concentration for 4 weeks and later. Group 5 was treated

with deionized water at week 1, 0.87 ppm at week 2, 3 ppm at week 3, 30 ppm at week 4, 300 ppm concentration at week 5 and later. No animals died and none of the toxic signs that could be seen were seen.

### Nucleic Acid Preparation

Liver mRNA expressions of HSP70 (Hspa1a, Hspa4, Hspa5), Hsp90 (Hsp90aa1) were evaluated using real-time RT-PCR analyses. Liver samples were collected from 15 rat at the end of day 100. Total RNA was extracted from the liver using trisol, chloroform, isopropyl alcohol, 70% ethanol, DPEC (Diethyl pyrocarbonate) according to the manufacturer's protocol. The concentration and quality of total RNA were estimated by spectrophotometry (absorbance at 260 nm). RNA was reverse transcribed to cDNA in a reaction mixture using Transcriptor First Strand cDNA Synthesis Kit - Roche. The cDNA was then used for Semi-quantitative real-time RT-PCR using Applied Biosystems® 7500 Real-Time PCR Systems.

Semi-quantitative real-time RT-PCR was performed using the TaqMan Prob.

### Primer Design and Synthesis

All the primers used in this study were synthesized by Shanghai Invitrogen Biotech Co Ltd. (Invitrogen, Shanghai) and these are listed in Table 1. According to the conserved sequences from known testes-specific Hsp70s and used to amplify target fragments of the testes-specific Hsp70 genes. RNA was used as the gene material and Hspala, Hspa4, Hspa5, Hsp90aa1 were used as primers. The nucleotide sequences of the mRNA data of NM 031971, NM 153629, NM 013083, and NM 17576102 were obtained from the gene bank of the internet (Anonym, 2012). By the rat Hsp70 and Hsp90 genes (Anonym, 2012). Were used to generate primers specific for the maximum of 1000 bp of the genes. The generated primers were checked using specific site specificities (Anonym, 2012).

**Table.1.** Primer and prob sequence.

<b>Hspa1a</b> / Forward Primer	5'-ACGCTGGCTGAGAAAGAGG-3'
<b>Hspa1a</b> / Reverse Primer	5-'ATCCACCTCCTCGATGGTG -3'
<b>Hspa1a</b> / TaqMan	FAM-TGCACAAGCGGGAGGAGCTG <sup>-TAMRA</sup>
<b>Hspa4</b> / Forward Primer	5'-TGCTTGAAAGGCTGAGTGG-3'
<b>Hspa4</b> / Reverse Primer	5'-TGGGAATAGAGGGCAATGAG-3'
<b>Hspa4</b> / TaqMan	FAM-TGGGACTGTGGGGCTGGTGC <sup>-TAMRA</sup>
<b>Hspa5</b> / Forward Primer	5'-ATTCCTGCGTCCGGTGTATTC-3'
<b>Hspa5</b> /Reverse Primer	5'TGGACGTGAGTTGGTTCTTG-3'
<b>Hspa5</b> / TaqMan	FAM-ACGATCAGGGCAACCGCATC <sup>-TAMRA</sup>
<b>Hsp90aa1</b> /Forward Primer	5'-CGAGAGCTTGACCGACCCTAG-3'
<b>Hsp90aa1</b> / Reverse Primer	5'-ATTCCAATGCCAGTATCCAC-3'
<b>Hsp90aa1</b> / TaqMan	FAM-TGGACTCGGGGAAGGAGCTGC <sup>-TAMRA</sup>

### Organ distribution of Hsp70 and Real-time RT-qPCR Analysis

The organ-dependent Hsp70 mRNA expression was measured real-time RT-qPCR as the following method. Briefly, first-strand cDNA was prepared as described above. Gene-specific primers (Q-F and Q-R; Table 1) were designed based upon the cloned Hsp70 cDNA to produce amplicons in different sizes. All RT-qPCR reactions were performed in triplicate using extracted RNA (pooled) of the

same concentration. Real-time RT-qPCR was performed in a C1000™ Thermal Cycler (Applied Biosystems® 7500 Real-Time PCR Systems) according to the manufacturer's instructions. The final volume of each RT-qPCR reaction was 25 µL, which contained 12.5 µL TaqMan prob master mix, 2.5 µL of diluted cDNA template, 8.7 µL of PCR-grade water, and 0.25 µL of each 10 µM primer. PCR conditions were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and a 0.5 °C/5 s incremental increase

from 60 °C to 95 °C that lasted 30 cycles. Hsp70 expression levels were calculated by the  $2^{-\Delta\Delta Ct}$  comparative Ct method (Livak and Schmittgen, 2001). Mean and standard deviations were calculated from triplicate experiments, and presented as the n-fold differences in expression relative to 18S rRNA.

### Statistics

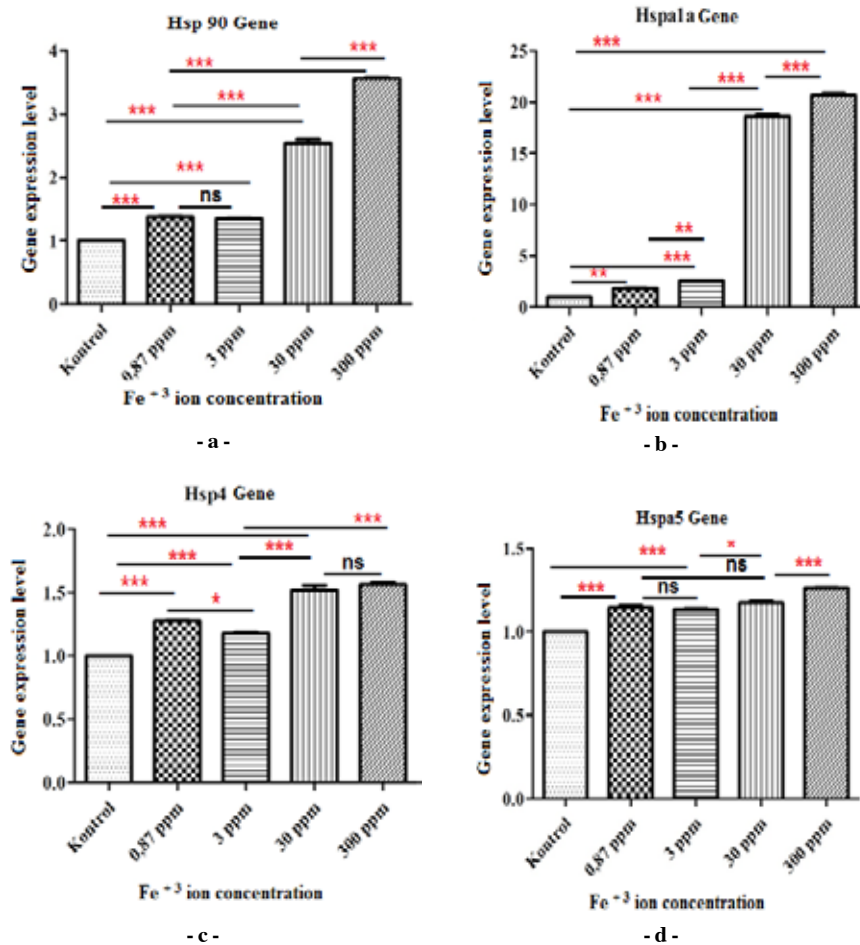
All statistics were analyzed by ANOVA method and variance analysis in SPSS 17.0 package program. Duncan multiple comparison test was used to determine the difference between significant group means as a result of ANOVA test and variance analysis. A p value of less than 0.05 was considered statistically significant. All values are shown as Mean  $\pm$  SDM (Mean  $\pm$  Standard Deviation) (n = 3).

## RESULTS AND DISCUSSION

### Gene Expression Results

Hsp90a1 gene expression in the liver tissue of rats exposed to different doses of heavy iron metal was found

to be statistically higher at 300 ppm than at the control at 0.87 ppm, 3 ppm, 30 ppm and 300 ppm treatment groups (Figure 1.a). There were statistically significant differences between the levels of Hspa1a mRNA of liver tissues exposed to sublethal doses of iron heavy metals in rats. In the 0.87 ppm, 3 ppm, 30 ppm and 300 ppm groups,  $p < 0.001 = ***$ , control and 0.87 ppm were compared and 0.8 ppm and 3 ppm  $p < 0.01$  were found to be significant (Figure 1.b). When the gene expression of Hspa4 in liver tissue was examined, it is seen that the differences between 0.87ppm and 30 ppm control are significant (0.87 ppm and 3 ppm:  $p < 0.05 = *$ , 30 ppm and 300 ppm:  $p > 0.05 = ns$ ) (Figure 1.c). When group comparisons in liver tissue of *Rattus norvegicus* were examined in general, the differences between gene expressions of all groups in relation to the control group were found to be significant 0.87 ppm and 3 ppm comparative and 0.87ppm and 30ppm comparative  $p > 0.05 = ns$ , 3ppm and 30ppm compared  $p < 0.05 = *$  with other groups except these, the change between the gene expression  $Fe^{3+}$  ion concentration was found statistically significant compared to the control.  $p < 0.001 = ***$  (Figure 1.d)



**Figure 1.** Gene expression levels of Hsp90, Hspa1a, Hsp4, Hspa5 in the liver are expressed as red stars, which are the p values obtained from Tukey's Multiple Comparison Test analysis. The number of stars varies from 1 to 3 in proportion according to the degree of importance. \* $p < 0.05 = *$ ,  $p < 0.01 = **$ ,  $p < 0.001 = ***$

While many studies have been done on HSP, these studies are mostly the effects of heavy metals, temperature, saltiness, stock intensity, competition or even calorie restriction on the expression of viable HSP genes. The effects of environmentally important heavy metals and organochlorines on the transcriptional profiling of genes encoding heat shock cognate 70 (Hsc70) and inducible heat shock protein 70 (Hsp70) in the tubercle fibroblast cell line were investigated. Specific reverse transcriptase polymerase chain reaction (RT-PCR) was used to test the effects of heavy metals (Cd<sup>+2</sup>, Cu<sup>+2</sup> and Ni<sup>+2</sup>) and organochlorines (aroclor 1254, hexachlorobenzene and 2-4-dichloroaniline) on the cell stress response. Hsp70 expression was induced in fibroblasts when exposed to concentrations of heavy metals as low as 0.01 µM, whereas expression of Hsc70 expression was induced when exposed to concentrations as low as 0.001 µM organochlorine. These studies show that gene members of the Hsp70 family are sensitive to environmental considerations (Deane et al., 2006). The distribution of heavy metals in various tissues of *Chanos chanos* collected from dirty regions and the associated oxidative stress have been studied comparatively to the fish collected from the less polluted regions of Kaattuppalli Island. Concentrations of copper, lead, zinc, cadmium, manganese and iron were measured in gills and liver. It is a highly injured work to prevent the detection of Hsp70 biomarkers for heavy metal induced oxidative stress and the heavy metal pollution that may accumulate in the future (Sivakumar et al., 2013). In our study, we tried to determine the effect of HSP gene expression in *S. dawley* rabbits exposed to Fe<sup>+3</sup> ion. It has been reported that HSP gene expression changes in response to heat shock depending on age and season (Murtha et al., 2003). As a matter of fact, in summer and winter the level of Hsp70 is high in the liver and in the spring (May) this level has fallen and the water has been exposed to various pollutants (Köhler et al. 2001). In a study conducted on oysters, it was reported that Hsp70 was more expressed in the autumn months (Encomio et al., 2005). The effects of heat shock and heavy metals have been extensively studied in all studies with this gene. Due to the various metabolic functions of different organs under the influence of heat stress, the level of Hsp 70 mRNA expression varies significantly in different tissues. Hsp 70 mRNA expression is higher in tissues such as heart and liver. Because these tissues have high metabolic

activity, oxidative stress produces free radicals. Oxidative stress increases the expression of Hsp 70 due to damage of liver cells. There are cases where heavy metal application reduces Hsp 70 level. For example, in a study of broiler chickens, the supplementation of the organic form of Se, Cr and Zn resulted in low Hsp 70 mRNA expression levels (Yahav 2009). Different temperatures were applied to different fish species, and in general, an increase in the exoplasmicity of this HSP family was recorded (Krone et al., 1997; Palmisano et al., 2000; Mesa et al., 2002; Murtha et al., 2003; Bowen et al., 2006). Another criterion applied to investigate the expression of the HSP group is heavy metals and various pollutants, and various concentrations were examined by applying to fish groups (Ait-Aissa et al., 2000; Boone and Vijayan 2002; Feng et al., 2003) and the increase in HSP expression was recorded in all of the applied doses.

In a study with mature zebrafish, the fish were exposed to 37 ° C temperature and RNA was isolated from different tissues and compared with Hsp expression using RT-PCR. Although Hsp70 expression showed a steady increase in brain, liver, and muscle, Hsp47 increased only in the brain, Hsp90 α-β and heat shock factor 1 (Hsf1a) were prominent in all three tissues, but the temperature did not increase in response to stress. In comparison with HSP expression, they observed an increase in basal Hsp70 and Hsf1a levels compared with younger adults. They also noted that there may be age differences in response to heat shock and mature zebrafish are suitable models for the aging process for Hsp studies (Murtha et al., 2003)

In our study, iron ions (0.87 ppm, 3 ppm, 30 ppm, 300 ppm) were given to Sprague-Dawley male rats of the male *Rattus norvegicus* strain and the HSP gene was monitored for exoplasm and an increase in HSP expression of iron ion was observed at different concentrations. Densities of the resulting bands were measured and an increase in HSP expression was observed due to the increase in iron concentration. According to the obtained results, changes in the expression levels of the Hsp70 (Hspa1a, Hspa4, Hspa5) and Hsp90 (Hsp90aa1) genes were detected by Real Time PCR method. HSP90aa1 and Hspa1a expression levels of HSP at 30 ppm and 300 ppm in the gene expression levels were significantly increased compared to the control (p<0.001=\*\*\*). There was

an increase in Hspa5 and Hspa4 gene expression levels when 3 ppm iron concentration was applied. When group comparisons in liver tissue of *Rattus norvegicus* were examined in general, differences between gene expressions of all groups were found to be significant according to the control group. The changes in Hspa5 and Hsp90aa1 gene expression levels between 0.87ppm and 3ppm iron ion exposure groups were compared between 0.87ppm and 30ppm iron ion exposure groups ( $p > 0.05 = ns$ ). A significant correlation was found between the results of 3ppm and 30ppm concentration application ( $p < 0.05 = *$ ). The other groups were statistically significant ( $p < 0.001 = ***$ ) compared to the control group between the 30 ppm and 300 ppm iron ion-treated Hspa4 gene expression levels compared to the other groups. Expression of these stress proteins can vary with many psychological, pathological, and age factors (Murtha et al., 2003). The work done by these stress proteins, which are molecular protectors in all organisms, is not limited to fish only, but has been tested in all organisms from man to mouse.

Mice exposed 4-7 and 22-28 month old mice to temperature stress at 42.5 ° C for 30 minutes and HSP70 expression was found to be 40-50% less than in young mice in the elderly. In another study with mice, temperature exposure observed increases in HSP and protein expression with age in neurons (Pardue et

al., 1992), liver and heart (Locke and Tanguay, 1996). Considering the physical and chemical conditions that are effective in HSP expression, it is necessary to better adjust the ambient conditions to remove the stress factors of the fish from the single environment. We studied how iron stress applied on mice affected the expression of heat shock proteins. As a result, we have examined comparatively which iron concentration influences which of the HSP genes we work most.

## CONCLUSION

As a result, we observed that among the genes that we examined expression change, the highest increase and change in the mice exposed to 300 ppm iron iodine occurred in the expression of Hspa1a gene. This study is an important study in determining the effect of stress on the expression of HSP genes in living organisms.

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## REFERENCE

- Ait-Aissa S, Porcher J, Arrigo A, Lambre C, 2000. Activation of the hsp70 promoter by environmental inorganic and organic chemicals: relationships with cytotoxicity and lipophilicity. *Toxicology*, 145:147–157.
- Anonym ,2012. <http://www.ncbi.nlm.nih.gov/>. (Erişim tarihi: 18 Eylül, 2012).
- Anonym, 2012. [http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi/](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi/).(Erişim tarihi: 18 Eylül, 2012).
- Anonym, 2012. <http://www.ncbi.nlm.nih.gov/BLAST/> . (Erişim tarihi: 18 Eylül, 2012).
- Boone AN, Vijayan MM, 2002. Constitutive heat shock protein 70 (HSC70) expression in rainbow trout hepatocytes: effect of heat shock and heavy metal exposure. *Comp Biochem Physiol C Toxicol Pharmacol* , 132(2):223-33.
- Bowen ID, Mullarkey K, Morgan SM, 1996. Programmed cell death in the salivary gland of the blow fly *Calliphora vomitoria*, *Microsc. Res. Techniq*, 34:202–217.
- Deane EE, Zhou L, Woo NY, 2006. Cortisol can be pro- or anti-apoptotic in sea bream cells: potential role of HSP70 induction for cytoprotection. *Mol Cell Endocrinol*, 259:57–64.
- Eddie E, Deane EE , Woo NY, Norman YS, 2006. Impact of heavy metals and organochlorines on *hsp70* and *hsc70* gene expression in black sea bream fibroblasts. *Aquatic Toxicology Amsterdam, Netherlands*, 79(1):9-15.
- Encomio VG , Stickler SM, Allen SK And Chu FL, 2005. Performance of “natural dermo-resistant” oyster stocks – survival, disease, growth, condition and energy reserves. *Journal of Shellfish Research*, 24: 143- 155.
- Heydari AR, Wu B, Takahashi R, Strong R, Richardson A, 1993. Expression of heat shock protein 70 is altered by age and diet at the level of transcription. *Mol Cell Biol*, 13: 2909–2918.
- Jolly C, Morimoto RI, 2000. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *Journal of the National Cancer Institute*, 92: 1564–1572.
- Keller JM, Escara Wilke F, Keller ET, 2008. Heat stress-induced heat shock protein 70 expression is dependent on ERK activation in zebrafish (*Danio rerio*) cells. *Comparative Biochemistry and Physiology*, 150: 307-314
- Köhler HR, Triebskorn R, Stöcker W, Kloetzel PM, Alberti G, 1992. The 70 kD heat shock protein (hsp 70) in soil invertebrates: a possible tool for monitoring environmental toxicants. “*Arch Environ Contam Toxicol*”, 22: 334–338.

- Krone PH, Lele Z, 1997. Hsp47 and Hsp70 gene expression is differentially regulated in a stress-and tissue-specific manner in zebrafish embryos. *Cell. Mol. Life Sci* Locke M, Tanguay RM ,21(2):123-33.
- Lindquist S, Henikoff S, and Meselson M, 1975. Localization of RNA from heat-induced polysomes at puff sites in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci*, 72: 1117-1121.
- Livak KJ, Schmittgen TD., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2<sup>-ΔΔC<sub>t</sub></sup> Method. *Elsevier Science (USA)*, 25(4):402-8.
- Marius L, Robert M,1996. Tanguay Increased HSF activation in muscles with a high constitutive HSP 70 expression. *Cell Stress Chaperones*,1: 189–196
- Mathew A, Morimoto RI, 1998. Role of the heat-shock response in the life and death of proteins. *Ann NY Acad Sci*, 851:99-111.
- Mesa MG, Weiland LK, Wagner P, 2002. Effects of acute thermal stress on the survival predator avoidance, and physiology of juvenile fall Chinook salmon. *Northwest Sci*, 76: 118–128.
- Mezquita B, Mezquita J, Durfort M, 2001. Constitutive and heat-shock induced expression of Hsp70 mRNA during chicken testicular development and regression. *J. Cell. Biol*, 82 (3): 480-490.
- Murtha JM, Keller ET, 2003. Characterization of the heat shock response in mature zebrafish (*Danio rerio*). *Exp Gerontol*, 38:683–691.
- Palmisano AN, Winton JR, Dickhoff W.W, 2000. Tissue specific induction of Hsp90 mRNA and plasma cortisol response in chinook salmon following heat shock, seawater challenge, and handling challenge. *Marine Biotechnology*, 2: 329-338.
- Pardu ML, Ballinger DG, Hogan NC, 1992. The heat shock response. Cells coping with transient stress. *Annals of the New York* 125–138 p.
- Sivakumar R, Jayprakash M , Natesan M, 2013. Effects of heavy metals on antioxidants and expression of HSP70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, Chennai, India. *Ecotoxicol Environ Saf*, 98:8-18.
- Sreedhar AS, Csermely P, 2004. Heat Shock Proteins In The Regulation Of Apoptosis: New Strategies In Tumor Therapy. *A Comprehensive Review.Pharmacology And Therapeutics*, 101: 227-257.
- Yan LJ, Christians ES, Liu L, Xiao X , Sohal R.S , Benjamin IJ , 2002. Mouse Heat Shock Transcription Factor 1 Deficiency Alters Cardiac Redox Homeostasis And Increases Mitochondrial Oxidative Damage. *The Journal of Embo*, 21: 5164-5172.
- Yahav S, 2009. Alleviating heat stress in domestic fowl: different strategies. *World's Poult Sci J*, 65:719–732.
- Zuhal U, 2009. *Pseudomonas Sp. Suslarında Cold Shock Protein İzolasyonu Ve Sds–Page Analizi. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi*, 80s.