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*Full Length Research Paper*

# Investigation of the protective effects of Vitamin C, iron and desferrioxamine on heat stress resistance in the model organism *Caenorhabditis elegans*

**Hasan Kılıçgün[1]*, Abdullah Olgun[2] and Zeynep Köksal[3]**

[1]Department of Nutrition and Dietetic, School of Health, Erzincan University, 24100 Erzincan, Turkey.
[2]Biochemistry Laboratory, Erzincan Military Hospital, Erzincan, Turkey.
[3]Graduate School of Applied Sciences, Erzincan University, Erzincan, Turkey,

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**The aim of this study was to determine the effects of Vitamin C (Vit. C), iron, the iron chelating agent desferrioxamine (DF) and their most effective dose combinations on thermotolerance of *Caenorhabditis elegans* (*C. elegans*). Due to the fact that studies on aging and lifespan take long time, thermotolerance was used as a first step analysis for using the time more effectively. The data of this study showed that when compared with the control group, the use of vitamin C, DF and iron alone or their combinations were associated with an increase in the survival of *C. elegans* under heat shock. However, the treatment with DF and its combinations resulted in a survival rate significantly higher than the other combinations. The results of our study suggested that under conditions of heat stress, treatment of DF has the most significant beneficial effect on survival of *C. elegans*, suggesting some possible pleiotropic mechanisms other than iron chelation.**

**Key words:** Thermotolerance, vitamin C, iron, desferrioxamine, aging.

## INTRODUCTION

The major natural antioxidants can be biologically compartmentalized depending on where they are found. Vit C is found in the cytosol and extracellular fluids and it readily scavenges the reactive oxygen and nitrogen species. Another important biological function of Vit C is its interaction with redox active transition metal ions such as iron and copper (Halliwell, 1996; Buettner and Jurkiewicz, 1996). The control of the levels of free transition metals such as iron and copper ions can help in preventing reactive oxygen species (ROS) formation. They are located in metal binding proteins such as ferritin, transferrin and ceruloplasmin. Iron is an essential element required for growth and survival of most organisms. The importance of iron is implicit in the role it plays in oxygen transport and heme synthesis as well as its ability to serve as a cofactor for enzymes involved in a variety of biological processes including DNA synthesis, energy production and neurotransmitter synthesis. Abnormally high concentration of cellular iron is toxic due to its ability to catalyze the generation of free radicals that damage DNA, lipids and proteins. In humans, the accumulation of excess cellular iron can result in cirrhosis, arthritis, cardiomyopathy, diabetes mellitus, and increased risk of cancer and heart disease. The concentration of intracellular iron is tightly controlled in order to provide adequate iron for cellular needs and prevent its accumulation (Benedetti et al., 2008). DF is a natural siderophore isolated from *Streptomyces pilosus*, whose function is to solubilize environmental iron by chelation and to transport it into the cell. It is a linear trihydroxamic acid that forms a kinetically and thermodynamically stable complex with iron (III), ferrioxamine (Corsi Ann, 2006; Brett et al., 2003). The mesylate salt is commonly used as a therapeutic agent (Desferal) for the removal of iron in states related to iron overload such as thalassemia.

*Corresponding author. E-mail: hkilicgun@hotmail.com. Tel: +90446 226 58 61/11507. Fax: +90446 226 58 62.

**Abbreviations: Vit. C**; Vitamin C; **DF,** desferrioxamine.

Although, DF has been viewed primarily as a metal chelating agent that blocks iron-dependent hydroxyl radical ($^{\bullet}OH$) formation via the Fenton or Haber–Weiss reactions (Brett et al., 2003), additional or alternative antioxidant mechanisms may operate in some of the models. Among these, direct reactions with oxidizing intermediates have been proposed. Indeed, desferrioxamine is also capable of scavenging free radicals, such as superoxide ($O_2^{\bullet-}$), $H_2O_2$, $^{\bullet}OH$ and peroxyl radicals (Tamas , 1998; Silvina et al., 2004; Meulenbelt et al., 1993; Pedchenko and Le Vine,1998; Auer et al., 2000; Hoe et al., 1982; Green et al., 1993), as well as oxoferryl compounds (Velsor et al., 2003). This experimental study was undertaken to assess the effect of Vitamin C, iron, DF and their combinations on thermotolerance of the nematode *C. elegans*. This organism is readily available, easy to culture in the laboratory, has a short lifespan, and vast knowledge is known of this nematode. Its entire genome and cell lineage is known.

## MATERIALS AND METHODS

The *C. elegans* wild-type (N2) strain and its food source *E. coli* OP50 strain were obtained from Caenorhabditis Genetic Center at the University of Minesota, (USA). *C. elegans* cultivation media supplies, Vitamin C and iron were purchased from MERCK (Germany), and deferoxamine mesylate (commonly known as desferrioxamine) was kindly provided by Novartis Institutes for Bio Medical Research from Switzerland. The survival analysis experiments were performed according to the standard protocol recently described by Sutphin and Kaeberlein (2009) except the concentrated OP50 bacteria were heat killed by incubating at 65°C. Additionally, the animals were kept at 25°C during growth into adult stage and then treated for one day with the agents tested until the thermotolerance assay. Thermotolerance assays were performed at 35°C until all the animals died by checking the animals at each hour. The escaping animals from the Petri dishes were excluded from the study. The substances iron (Fe III), Vit C and DF were added to both the food source and nematode growth medium (NGM). This study was planned as six groups of worms and each experiment was done in replicates of three. One group was used as control. The others were; iron (Fe III) (6.6 mg/ml), Vit C (80 mg/ml), DF (100 µM), Vit C + DF and Vit C + Fe III. The final concentrations were chosen according to the previous literature (Brett et al., 2003; Gita, 2008). Worms were incubated at 35°C. Animals which were in different groups were counted and recorded at the beginning of each hour.

### Statistics

Data were presented as mean ± S.D. of at least three independent experiments. One-way ANOVA followed by Scheffe's test were performed to determine statistical differences between groups with the aid of SPSS software version 11.0 (SPSS, Chicago, IL, USA). Statistical significance was defined as $p < 0.01$ and $p < 0.05$ for all tests.

## RESULTS AND DISCUSSION

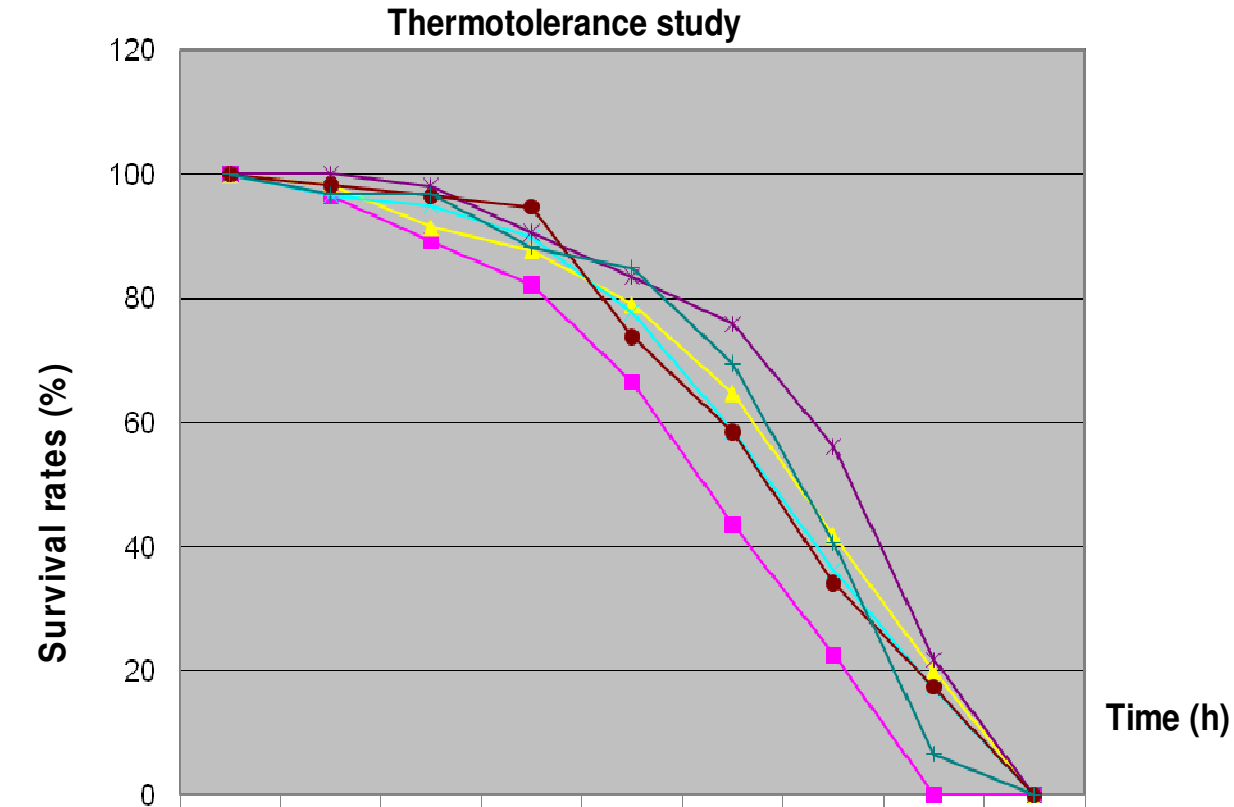
Thermotolerance is an increased resistance of cells, tissues and organisms to elevated temperatures. The measurement of thermotolerance has been used with a specific aim to reflect lifespan. The reason for this is not only because thermotolerance assays are easy to implement and give consistent results, but also because heat shock response acts through an evolutionarily highly conserved biological mechanism that is present in almost every living system studied (Verbeke et al., 2001). It helps in physiological adaptability against not only heat but a number of metabolic stresses and toxicants (Tiwari et al., 1995; Petersen, 1990). All living systems have the intrinsic ability to respond, to counteract and to adapt to the external and internal sources of disturbance (Suresh and Rattan, 2008). Thermotolerance has been demonstrated in cell lines, tissue culture, and in several animal species. In *C. elegans*, resistance to high temperature is often correlated with increased lifespan (Dana and Mark, 2007).

Although, Vit C is a very powerful *in vitro* antioxidant, sometimes it does not exhibit this effect *in vivo.* This is likely a result of its prooxidant activity. As a matter of fact, it was shown to have damaging effect on DNA (Seon et al., 2001). Seon et al. (2001) determined that Vit C induces lipid hydroperoxide decomposition to the DNA-reactive bifunctional electrophiles 4-oxo-2-nonenal, 4, 5-epoxy-2(E)-decenal, and 4-hydroxy-2-nonenal. The compound 4, 5-Epoxy-2(E)-decenal is a precursor of etheno-2'-deoxyadenosine, a highly mutagenic lesion found in human DNA. Therefore, its damaging effects might be counteracting its beneficial effects and provide a possible explanation for its ineffectiveness *in vivo*. Since transition metals like iron enhance the prooxidant activity of Vit C (Anitra and Balz, 1999), their absence or decrease can enhance the antioxidant activity of vitamin C. So, in order to see the effect of Vit C on the thermotolerance of *C. elegans*, we decided to analyse Vit C in a condition with absent or lowered iron. For this purpose, DF was used to chelate iron. In some previous studies, the effects of Vit C and iron were tested on the lifespan of *C. elegans*. In this study, it was shown that high concentrations of iron did decrease the lifespan of worms and different dose combinations of Vit C and Vit E had positive effect on lifespan of *C. elegans* (Gita, 2008). Additionally, we wanted to answer the questions:

1. Do Vit C and iron have a positive effect on the survival of *C. elegans* under heat shock?
2. Is it better to use the combination of Vit C and iron or does using one alone have the same impact?
3. Does Vit C has a better effect in the presence of DF, in other words in the absence of iron?

In order to answer these questions, we analysed thermotolerance of the worms in the presence and absence of these agents.

Vit C exhibited a protective effect against heat stress. The survival rate of vitamin C group was significantly higher than that for the control group. Vit C in combina-




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Control | 100 | 97 | 89 | 82 | 66 | 44 | 22 | 0 | 0 |
| Fe | 100 | 98 | 91 | 88 | 79 | 65 | 42 | 20 | 0 |
| VitC | 100 | 97 | 95 | 90 | 78 | 59 | 36 | 17 | 0 |
| DF | 100 | 100 | 98 | 91 | 83 | 76 | 56 | 22 | 0 |
| Vitc+DF | 100 | 98 | 96 | 95 | 74 | 58 | 34 | 17 | 0 |
| VitC+Fe | 100 | 97 | 97 | 88 | 85 | 69 | 41 | 7 | 0 |

**Figure 1.** The effect of Vitamin C, iron and desferrioxamine on the thermotolerance of *C. elegans*.

tion with DF and iron also showed a protective effect against heat stress when compared with the control group (Figure 1; Table 1). Iron and its combination with DF provided a strong protective effect against heat stress. But the protective activity of the iron was significantly higher than its combinations (Figure 1, Table 1). Some recent studies have shown that various vitamins, and macro and micro-minerals, including iron, iodine, fluorine, selenium, zinc and copper have beneficial effects which are considered to be achieved through stress response induced alterations in gene expression of various maintenance and repair pathways (Verbeke et al., 2001; Hayes, 2007; Mocchegiani et al., 2006; Benedetti et al., 2008). These studies can provide an insight into the mechanism of the positive effect we observed with iron. The treatment with DF alone and its combinations had also a better survival rate than the untreated animals in the control group. On the other hand, treatment with DF alone resulted in a survival rate most significantly higher than all other groups (Figure 1, Table 2). DF might be protecting the worms against heat shock through by altering the activity of signalling pathways involved in thermotolerance. The fact that DF transports iron inside the cell and change the intracellular and extracellular iron ratios needs to be addressed for the explanation of its effects. Given the beneficial effect of DF on thermotolerance, it is hoped that these important findings will be taken into account in all future studies. On the other hand, it would be interesting to test whether different doses of DF prevent aging in *C. elegans* and how this relates to the mechanism of action of DF in lifespan.

**Table 1.** Comparison of Vitamin C, iron and desferrioxamine with control in animal model *C. elegans*.

| Group | N | $\overline{X}$ | Sd | P |
|---|---|---|---|---|
| Control- Fe | 100 | 55.61 | 39.60 | 0.002* |
| | 100 | 65.04 | 35.73 | |
| Control- Vit C | 100 | 55.61 | 39.60 | 0.000* |
| | 100 | 63.44 | 36.39 | |
| Control- DF | 100 | 55.61 | 39.60 | 0.000* |
| | 100 | 69.54 | 35.90 | |
| Control- Vit C-DF | 100 | 55.61 | 39.60 | 0.001* |
| | 100 | 63.68 | 38.57 | |
| Control-Vit C-Fe | 100 | 55.61 | 39.60 | 0.000* |
| | 100 | 64.78 | 39.00 | |

*p < .01.

**Table 2.** Comparison of thermotolerance effects of Vitamin C, iron and desferrioxamine in animal model *C. elegans*.

| Group | N | $\overline{X}$ | Sd | P |
|---|---|---|---|---|
| Fe – Vit C | 100 | 65.04 | 35.73 | 0.393 |
| | 100 | 63.44 | 36.39 | |
| Fe - DF | 100 | 65.04 | 35.73 | 0.030** |
| | 100 | 69.54 | 35.90 | |
| Fe – Vit C-DF | 100 | 65.04 | 35.73 | 0.619 |
| | 100 | 63.68 | 38.57 | |
| Fe – Vit C-Fe | 100 | 65.04 | 35.73 | 0.932 |
| | 100 | 64.78 | 39.00 | |
| Vit C - DF | 100 | 63.44 | 36.39 | 0.002** |
| | 100 | 69.54 | 35.90 | |
| Vit C – Vit C-DF | 100 | 63.44 | 36.39 | 0.908 |
| | 100 | 63.68 | 38.57 | |
| Vit C – Vit C-Fe | 100 | 63.44 | 36.39 | 0.459 |
| | 100 | 64.78 | 39.00 | |
| DF – Vit C-DF | 100 | 69.54 | 35.90 | 0.046 |
| | 100 | 63.68 | 38.57 | |
| DF – Vit C-Fe | 100 | 69.54 | 35.90 | 0.018** |
| | 100 | 64.78 | 39.00 | |
| Vit C – Vit C-Fe | 100 | 69.54 | 38.57 | 0.629 |
| | 100 | 64.78 | 39.00 | |

**p < .05.

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