Life span effects of *Hypericum perforatum* extracts on *Caenorhabditis elegans* under heat stress

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ABSTRACT

Background: The beneficial effects of antioxidants in plants are mainly extrapolated from *in vitro* studies or short-term dietary supplementation studies. Due to cost and duration, relatively little is known about whether dietary antioxidants are beneficial in whole animals' life span or not. Materials and Methods: To address this question, under heat stress (35°C), *Hypericum perforatum* was extracted with petroleum ether and the nematodes *Caenorhabditis elegans* exposed to three different extract concentrations (1mg/mL, 0.1mg/mL, 0.01mg/mL) of *H. perforatum*. Results: We report that *Hypericum perforatum* extracts did not increase life span and slow aging related increase in *C. elegans*. Moreover, one fraction (1mg/mL) increased declines of C. elegans life span and thermotolerance. Conclusion: Given this mounting evidence for life span role of *H. perforatum* in the presence of heat stress in vivo, the question whether *H. perforatum* acts as a prooxidant or an antioxidant in vivo under heat stress arises.



Key words: Aging, Caenorhabditis elegans, Hypericum perforatum, life span, thermal stres

INTRODUCTION

Plant species of the genus Hypericum are well known for their use in traditional medicine due to the therapeutic efficacy of its many different species. One of the most important and recognised species of the genus is H. perforatum L., used in traditional and contemporary medicine to the high content of hypericins and hyperforins in its flowers, externally for the treatment of skin wounds, eczema and burns, and internally for disorders of the central nervous system, the alimentary tract and other purposes. [1,2] Especially, its antidepressant activity has been related to its phenolic composition and flavonoids suggesting that they could have important antioxidant properties. [3] It was suggested that the combination of antioxidant/antiinflammatory polyphenol compounds found in fruits and vegetables may show efficacy in reversing aging. In other words, dietary consumption of compounds in plants, fruits and vegetables can attenuate age-related declines in several physiological and functional indices.^[4] In aging studies it was also seen that if reactive free radicals are decreased or antioxidant substances are increased, the life span of

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Dr. Hasan Kılıçgün, Department of Nutrition and Dietetic, Erzincan University, School of Health, 24100, Erzincan, Turkey. E-mail: hkilicgun@hotmail.com the organisms can be increased.^[5,6] One of this organism which are used for life span studies is *Caenorhabditis elegans* which is readily available, easy to culture in the laboratory, has a short life span, and vast knowledge is known about this nematode. Moreover, studies have shown that some compounds can prolong *C. elegans* life span under heat stress and laboratory conditions.^[7,8] In the light of these studies, we aimed to determine the protective effects of different concentrations of *H. perforatum* on life span of *C. elegans* exposed to heat stress.

In this study, it can be reported that no encouraging results from *Hypericum* extracts were obtained when the inhibition of heat stress damage and longevity effect on *C. elegans* were put into consideration. Furthermore, high concentration promoted the heat stress damage and exhibited a functional pro-oxidant role. In conclusion, it is taken for granted that the complex composition of plant extracts can lead to contradictory results.

MATERIALS AND METHODS

The *C. elegans* wild-type (N2) strain and its food source *Escherichia coli* OP50 strain were obtained from Caenorhabditis Genetic Center at the University of Minesota, (USA). *C. elegans* cultivation media supplies were

purchased from MERCK (Germany), and H. perforatum was collected at different developmental stages (vegetative, floral budding, full flowering and seed set) from its wild habitat in Çayırlı, Erzincan province; specimen was deposited at the Herbarium of Medicinal Plants and Drugs Research Institute, Erzincan University, Erzincan, Turkey. H. perforatum used in this study was identified by Asisstant Professor Mustafa Korkmaz in Department of Biology, University of Erzincan. Aerial part of plant was air-dried in the dark room and then ground to powders using a mechanical grinder. Weighing 20 g homogenized sample was transferred to the extraction vessel and extracted with Soxhlet device in 300 ml of petroleum ether (Merck 1.01775.5000). Extraction was continued for approximately 16 hours. Following extraction, the extract was transferred to petri dish and placed in the oven at 37°C (Nüve N500). Therefore, petroleum ether was totally evaporated, the extract was diluted with distillate water when it was used for life span experiment. The survival analysis experiments were performed according to the standard protocol recently described by Sutphin and Kaeberlein, [9] except for the concentrated OP50 bacteria that were 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 by checking the animals at each hour until all the animals died. The escaping animals from the Petri dishes were excluded from the study. Different concentrations of H. perforatum were added to both the food source and nematode growth medium (NGM) consisting of 8.5 g bacto agar, 1.5 g sodium chloride, 1.25 g peptone, 0.5 ml cholesterol (5 mg/ ml stock prepared in 95% ethanol), 0.5 ml of 1 M CaCl, 0.5 ml of 1 M MgSO₄, 12.5 ml of 1 M KH₂PO₄, and 487.5 ml nanopure water per 500 ml NGM. The solidified NGM agar plates were spotted with OP50, a strain of E. coli used as a food source. The study was planned as four groups. One group was used as control. The others were 1mg/mL, 0.1mg/mL, 0.01mg/mL concentrations of H. perforatum respectively. Each experiment was done in replicates of three. Then worms were incubated at 35°C. Animals were counted and recorded on the hour.

Statistics

Data are presented as mean \pm 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 for all tests.

RESULTS AND DISCUSSION

A critical component of the homeodynamic property of

living systems is their capacity to respond to stress. In this context, the term "stress" is defined as a signal generated by any physical, chemical or biological factor (stressor), which initiates a series of events in order to counteract, adapt and survive in a living system. While a successful and overcompensatory responses to low doses of stressors improve the overall homeodynamics of cells and organisms, an incomplete or failed homeodynamic response leads to the damaging and harmful effects of stress, including death.^[10] Heat stress is one of those stresses that has been used with a specific aim to test and apply hormesis. The reason for this is not only because heat stress is easy to implement and gives consistent results, but also because heat stress mainly acts through an evolutionarily highly conserved stress response pathway known as the heat shock response.^[11] Effects of mild and severe heat stress have been tested on yeast, nematodes, fruit flies, and rodent and human cells. For example, a 2-h heat stress at 37°C applied before the first division and after the fourth division extended the replicative life span of Saccharomyces cerevisiae by 10%. [12] The same stress had no effect when applied in a later period of life as well as applied everyday. In the case of nematodes, wild-type and age-1 mutant hermaphrodite C. elegans exposed for 3-24 h to 30°C exhibited a significant increase in mean life span compared to controls.[13,14] Similarly, a 6 h exposure at 30°C of wild-type worms induced a 12.5% increase in life span, but no effect was found after exposures of 2 or 4 h. [15] In a study of multiple stresses in C. elegans, an extension of life span after 1 and 2 h heat stress at 35°C was reported. [14,16] In another study performed on C. elegans, it was observed that repeated mild heat treatments throughout life had a larger effect on life span compared to a single mild heat treatment early in life, and the effect was related to the levels of heat shock protein expression.^[17] Therefore, in this study, we aimed to determine the protective effects of different concentrations of H. perforatum on life span of C. elegans exposed to heat stress. In the concentration-dependent experiments, it was observed that there was not any statistically significance between survival rates of control group and 0.1 mg/mL and 0.01 mg/mL concentrations of Hypericum extract [Figure 1, Table 1]. As a matter of fact, in a series of articles, [18,19] the purpose was to observe the survival of C. elegans worms which were subjected to 35°C heat stress of different durations. Those studies showed that heat stress not longer than 2 h produced an extension of life span of animals. In contrast, longer heat stress had either no effect or deleterious effects. In a study of multiple stresses in C. elegans an extension of life span after 1 and 2 h heat stress at 35°C was reported.[14,16] These results are in agreement with findings of our study and seem to support our study. On the other hand, at the concentration of 1mg/mL, survival rates of worms are significantly lower than that

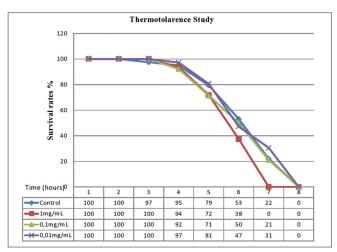


Figure 1: The life span effect of different concentrations of *H. perforatum* extracts on *Caenorhabditis elegans* under heat stres (35°C)

Table 1: Comparison of thermotolerance effects *Hypericum* concentrations, and control on animal model *C. elegans*

Groups	N	X	sd	P
Control -Hypericum 1 mg/mL	25	12.94	7.33	0.00*
	25	10.06	6.85	
Control - Hypericum 0,1 mg/mL	25	12.94	7.33	0.48
	25	12.69	7.24	
Control - Hypericum 0,01 mg/mL	25	12.94	7.33	0.33
	25	12.50	6.81	

asterisks (*) in the same line are statistically different, data is presented as group mean values $\pm\,\text{SD}.\,^*P\,<\,\text{o.ol}$

Table 2: Comparison of thermotolerance effects of *Hypericum* and its concentrations on animal model *C. elegans*

Groups	N	\overline{X}	sd	P
Hypericum 1 mg/mL-Hypericum	25	10.06	6.85	0.00*
0,1 mg/mL	25	12.69	7.24	
Hypericum 1 mg/mL-Hypericum	25	10.06	6.85	0.00*
0,01 mg/mL	25	12.50	6.81	
Hypericum 0,1 mg/mL-	25	12.69	7.24	0.64
Hypericum 0,01 mg/mL	25	12.50	6.81	

asterisks (*) in the same line are statistically different, data is presented as group mean values \pm SD. *P < 0.01

of control group [Figure 1, Table 1]. This effect may be attiributed to polyphenol compounds of *H. perforatum*.^[3] As a matter of fact, some studies have reported that the same polyphenol compounds could behave as both antioxidants and prooxidants depending on concentration and free radical source.^[20,21] However, to the best of our knowledge, no detailed study concerning dose properties

and the toxic effects of *H. perforatum* on life span of *C. elegans* has been performed so far. This may be the first study to provide data on this matter. The inter groups test showed that the longevity effect of variable groups were not statistically significant between the group of 0.1mg/mL and 0.01mg/mL except for 1mg/mL. In other words, the group of 1mg/mL showed a more significant decrease in lifespan of *C. elegans* [Figure 1, Table 2].

Finally, the thermotolerance activity on C. elegans life span was not related to the 0.01mg/mL, 0.1mg/ mL concentrations of Hypericum extracts. Also, the activity was not increased significantly as a result of increasing concentration. Moreover, the thermotolerance activity dramatically decreased at the highest (1mg/mL) concentration [Figure 1, Tables 1 and 2]. In summary, the results from the present in vivo study demonstrate that H. perforatum does not promote thermotolerance activity. Moreover, instead of acting as an antioxidant towards heat stress in vivo, it has showed hazardous activity on life span of C. elegans in the presence of high concentration and has had not the potential to be used as an anti-aging agent. Also, these results suggest that the same plant that optimizes antioxidant capacity may also act as a prooxidant in different test systems depending on its concentration.

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