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PHYSIOLOGICAL AND LIFESPAN ALTERATIONS IN *CAENORHABDITIS ELEGANS* EXPOSED TO BENZOIC ACID

Hülya ÖZPINAR[1,] , Necati ÖZPINAR[2], Hasan KILIÇGÜN[3], Şeker DAĞ[4], Musa SARI[5]

[1]Faculty of Sciences, Department of Biology, University of Cumhuriyet
[2]Erzincan food control laboratory of the third army
[3]Department of Nutrition and Dietetic, School of Health, University of Erzincan
[4]Faculty of Sciences, Department of Biology, University of Cumhuriyet
[5]Faculty of Sciences, Department of Biology, University of Cumhuriyet

Abstract: Benzoic acid derivatives are widely used as industrial chemicals, agrochemicals, pharmaceuticals and consumer products. Even though benzoic acid is generally considered as safe, adverse effects such as asthma, urticaria, metabolic acidosis and convulsions were observed at low doses in sensitive persons. The objective of this experimental study was to evaluate the physiological and lifespan alterations effects of different levels of benzoic acid on model animal *Caenorhabditis elegans*. The findings were compared with the control group. The results showed that benzoic acid fractions (0.1 gr, 0.06 gr, 0.02 gr / 10 ml,) had adverse effects on lifespan fertility and growing properties of *Caenorhabditis elegans*. On the other hand, there were not any differences between control group level and 0.008 gr, 0.001 gr / 10 ml fractions of benzoic acid in terms of lifespan effect. However, the 0.008 gr, 0.001 gr / 10 ml groups showed much lower fertility and growing properties than control group under experimental conditions of this study. In conclusion, dietary benzoic acid affects lifespan and fertility of *Caenorhabditis elegans* in a dose dependent manner. Therefore, it is important to assess the influence of dietary benzoic acid addition in products and its concentration has to be controlled.

Keywords: Benzoic Acid, *Caenorhabditis elegans*, Lifespan, Fertility

BENZOİK ASİTE MARUZ BIRAKILAN *CAENORHABDİTİS ELEGANS*’ LARDA YAŞAM SÜRESİ ANALİZİ VE FİZYOLOJİK DEĞİŞİKLİKLER

Özet: Benzoik asit ve benzoik asitten üretilen maddeler kimya endüstrisi, tarım sektörü, ilaç endüstrisi ve bir çok tüketim ürünlerinde yaygın bir şekilde kullanılmaktadır. Benzoik asitin genellikle güvenilir olduğu düşünülmesine rağmen duyarlı kişilerde düşük dozlarda bile astım, ürtiker, metabolik asidoz ve konvülsiyonlar gibi yan etkileri olduğu görülmüştür. Bu çalışmada bir model organizma olan *Caenorhabditis elegans* farklı dozlarda benzoik asite maruz bırakılmış, fizyolojik ve yaşam süresi değişiklikleri incelenmiştir. Bulgular kontrol grubuyla karşılaştırılmıştır. Çalışma sonucunda benzoik asitin 0.1 gr, 0.06 gr, 0.02 gr / 10 ml dozlarında *Caenorhabditis elegans*' larda yaşam süresi, fertilite ve fiziksel büyüme üzerine olumsuz etkileri olduğu görülmüştür. Diğer taraftan 0.008 gr, 0.001 gr / 10 ml lik dozlarda yaşam süresi üzerinde kontrol grubuyla herhangi bir fark görülmemiştir. Fakat 0.008 gr, 0.001 gr / 10 ml dozlar fertilite ve fiziksel büyümenin kontrol grubuna gore daha az olduğu tespit edilmiştir. Sonuç olarak benzoik asitin doza bağlı olarak yaşam süresi, fertilite ve fiziksel büyüme üzerine olumsuz etkileri olduğu görülmüştür. Bu yüzden gıda ek maddesi olarak yaygın bir şekilde kullanılan benzoik asitin konsantrasyonunun kontrolü büyük önem taşımaktadır.

Anahtar sözcükler: Benzoik Asit, *Caenorhabditis elegans*, Yaşam Süresi, Fertilite

INTRODUCTION

Food additives play a vital role in today's food supply. A food additive is any substance or mixture of substances, other than basic food components, added to food in a scientifically controlled amount (Mpountoukas et al., 2008). These additives are used widely for various purposes, including preservation, colouring and sweetening. Some food additives,

however, have been prohibited from use because of their toxicity (Sasaki et al., 2002). Benzoic acid is a weak organic acid that resembles the mode of action of formic acid (Papadomichelakis et al., 2011). Its derivatives are widely used as industrial chemicals, agrochemicals, pharmaceuticals and consumer products. Even though benzoic acid is generally recognized as safe, adverse effects such as asthma, urticaria, metabolic acidosis and convulsions were observed at low doses in sensitive persons (Ping et al., 2009). Also, benzoic acid at higher than permitted safety levels can do harm to human health (Shan et al., 2007). The maximum permitted concentrations of benzoic acid in each type of food are controlled by legislation and the concentration of naturally occurring benzoic acid in several foods should not exceed an average value of 1.000 mg/kg of food. Also, maximum concentrations reported for benzoic acid added to food for preservation purposes were in the range of 2.000 mg/kg of food (Hamzah et al., 2011). *Caenorhabditis elegans* (*C. elegans*) has been commonly used as a model organism in recent years. It is a small worm, just 1 mm in length that lives in soil and has been used extensively for aging studies, mainly because of its short and consistent lifespan (average 14-20 days at 20^{o}C) and better characterized aging-related pathways than other organisms (Chuang et al., 2009). Also, considerable toxicity testing has been performed employing *C. elegans* as a bioassay system, various endpoints have been assessed in both aquatic and soil environments. Most widely used end points include lethality, fecundity and motility, reproduction, development, growth rate and inhibition of feeding (Leung et al., 2008, Williams et al., 1990, Rajini et al, 2008). Taking all this information into account, the objective of this study was to investigate physiological and lifespan alterations effects of different concentration of benzoic acid on model animal *C. elegans*.

MATERIALS AND METHODS

Bristol N2 (wild-type) *C. elegans* strain and *E. coli* OP50 were obtained from the Caenorhabditis Genetic Centre at the University of Minnesota. Quantization of constitutive egg-laying was performed according to the standard protocol described by Koelle (2005). Briefly 25 late adult *C. elegans* animals were picked from an unstarved plate and delivered a fresh plate. 36 hours later, 20 animals were picked to a fresh plate. In case transferring some eggs with the animals, the animals were let to crawl away from these eggs for a few minutes, and then the animals were picked again to a fresh plate. Especially this time, it was made sure that no eggs were transferred. The animals were set at 20°C for 30 minutes. In that time interval, there should have been 25-100 eggs on the plate. The number of eggs were remarked at the end of 30 min with a 20 X objective. To help scan across the plate systematically, parallel black lines drawn used to place the plate inside a plate lid with it. The study was repeated after 24 hours. Usually, the standard deviation between days is more like 20%. The life span analysis experiments were performed according to the standard protocol described by Sutphin et al. (2009) except the concentrated OP50 bacteria which were killed by incubating at 65^{o}C for 30 minutes. Benzoic acid (Merck) was added to both NGM and lawn of bacteria to allow complete exposure of animals. 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 tested final concentrations of benzoic acid were 0.1 gr/10 ml, 0.06 gr/10 ml, 0.02 gr/10 ml, 0.008 gr/10 ml, 0.001 gr/10 ml. The worms were grown at 20^{o}C. The escaping animals from the petri dishes were excluded from the study.

STATISTICAL ANALYSIS

Paired Samples T Test to determine statistical differences between groups with the aid of SPSS software version 11.0 (SPSS, Chicago, IL, USA). Statistical significance was defined as $P<0.05$ for all tests.

RESULTS AND DISCUSSION

The use of additives in food and liquid has increased immensely in the last decades. What is more, the main reason, given by food manufacturers for using additives is that food would spoil in a short time without additives (Burcu et al., 2009). To identify longevity, fertility and growing promoting properties of benzoic acid (E-210), which is commonly used in many food products for preserve these substances from yeast, mould and bacteria effects, wild-type *C. elegans* (N2) was used as a live model organism because the nematode *C. elegans* has proven to be a very useful experimental organism for the study of longevity. Also, more than 70 genes have been found to influence the lifespan in this organism (Chuang et al., 2009, Yılmaz et al., 2009). In this study, benzoic acid caused a decreasing effect on cracking of eggs, growing of nematodes and hatching percentage of new generation. This increase was significant at all concentrations. Those effects were dose-dependent especially at (0.1 gr / 10 ml, 0.06 gr / 10 ml, 0.02 gr / 10 ml) concentrations. The adverse effect potency of benzoic acid concentrations on cracking of eggs, growing and hatching percentage of new generation was higher than for the positive control (Figure 1-4). Benzoic acid decreased the lifespan especially at the highest concentrations compared with the control. Moreover this decrease was significant as statistically (Table 1, Figure 5). Benzoic acid decreased the lifespan a dose-dependent manner at the concentrations (0.1 gr / 10 ml, 0.06 gr / 10 ml, 0.02 gr / 10 ml). On the other hand, there were not any differences between control group level and 0.008 gr / 10 ml, 0.001 gr / 10 ml fractions of benzoic acid in terms of life span effect. However, the 0.008 gr / 10 ml, 0.001 gr / 10 ml concentrations showed much lower fertility (cracking of eggs and hatching) and growing properties than control group under experimental conditions of this study (Figure 1-5, Table 1).

In the present study, dietary benzoic acid affected lifespan, cracking of eggs, hatching percentage of new generation and growing properties of *Caenorhabditis elegans* with the effects depending on its concentrations. Moreover, the most striking observation in this study was the detection of maximal hazardous (0.1 gr / 10 ml) and minimal hazardous (0.001 gr / 10 ml) dosage intervals of benzoic acid affecting on the hatching percentage of new generation lifespan and growing properties in the animal model *C. elegans*. As a matter of fact, an extensive review of the toxicity of benzoate showed that at high doses it interferes with an intermediary metabolism, including the urea cycle, gluconeogenesis, fatty acid metabolism, and the tricarboxylic acid cycle (WHO., 1997). In addition to these results, there are arguments concerning additives in food. In this vein, some countries already limited some food products in schools because ingredients or additives of those products may cause health/behaviour problems in children (Wegrzyn et al., 2001, Fried et al., 2002). However, to our best knowledge, we could not coincide with studies on the effects of benzoic acids, as well as other carboxylic acids on lifespan fertility and growing properties of *C. elegans*. Whereas numerous studies have been made on the antibacterial, antifungal, and antiviral activities of natural and synthetic phenolic compounds including various substituted benzoic acids (Friedman ., 2003, Tfouni ., 2002). In the light of these results, this study can be the first to undertake an investigation of the cracking of eggs, hatching percentage of new generation lifespan and growing properties in the animal model *C. elegans* exposured to benzoic acid at different concentrations.

In conclusion, this study can be useful for the sustainable future use of this organic acid. Considering the infant populations and sensitive persons, the official authorities and manufacturers should establish the monitoring program of benzoic acid in order to get good quality products. On the other hand, further studies should be continued to get proper information regarding the role of benzoic acid as a food additive, to find the factors that influence the effects of benzoic acid in products and its involvement in the other dose-dependent processes.

Figure 1: The Percentage of Cracked Eggs in Day1

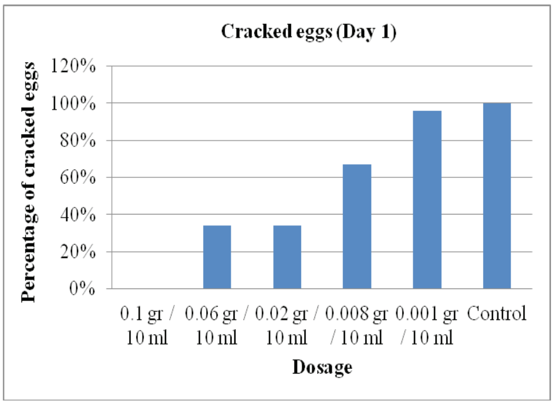

Figure 1 shows dose-dependent effects of 1-day dietary exposure to benzoic acid on cracking of eggs of *C. elegans.*

Figure 2: Growing Percentage of Nematodes from Cracked Eggs in Day 2

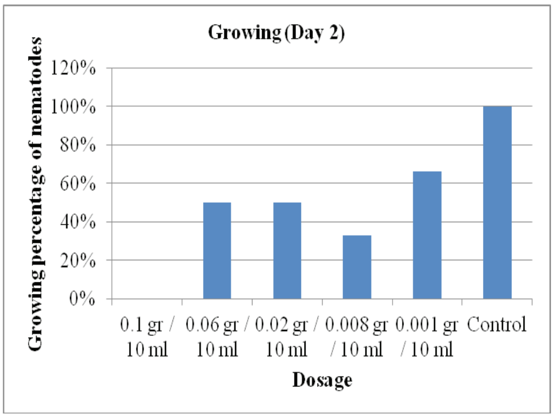

Figure shows dose-dependent effects of 2-day dietary exposure to benzoic acid on growing percentage of *C. elegans*

Figure 3: Hatching Percentage of New Generation Nematodes in Day 3

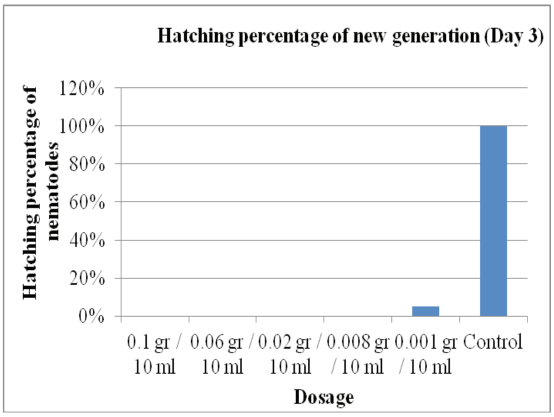

Figure 3 shows dose-dependent effects of 3-day dietary exposure to benzoic acid on hatching percentage of new generation of *C. elegans*

Figure 4: Hatching Percentage of New Generation Nematodes in Day 4

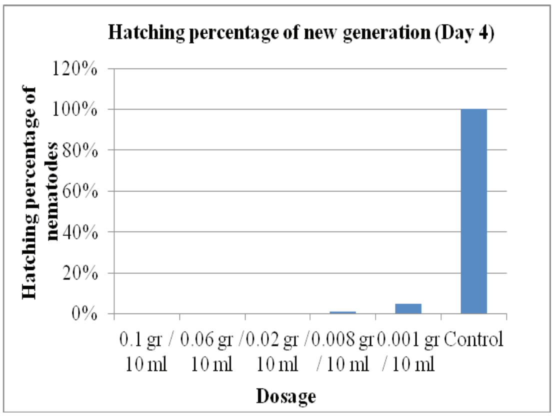

Figure shows dose-dependent effects of 3-day dietary exposure to benzoic acid on hatching percentage of new generation of *C. elegans*

Table 1: Comparison of Lifespan Effects of Benzoic Acid and Its Concentrations in Animal Model *C. elegans*.

Groups	Mean ± Std. Deviation	p
CONTROL – 0.001gr/10ml	0.70 ± 1.89	0.115
CONTROL – 0.008gr/10ml	0.50 ± 2.21	0.325
CONTROL – 0.02gr/10ml	2.05 ± 2.09	0.000*
CONTROL – 0.06gr/10ml	4.25 ± 2.24	0.000*
CONTROL – 0.1gr/10ml	5.20 ± 2.88	0.000*
0.1gr/10ml – 0.06gr/10ml	0.95 ± 1.39	0.007*
0.1gr/10ml – 0.02gr/10ml	3.15 ± 2.01	0.000*
0.1gr/10ml – 0.008gr/10ml	4.70 ± 3.57	0.000*
0.1gr/10ml – 0.001gr/10ml	4.50 ± 3.49	0.000*
0.06gr/10ml – 0.02gr/10ml	2.20 ± 1.58	0.000*
0.06gr/10ml – 0.008gr/10ml	3.75 ± 2.42	0.000*
0.06gr/10ml – 0.001gr/10ml	3.55 ± 2.35	0.000*
0.02gr/10ml – 0.008gr/10ml	1.55 ± 2.11	0.004*
0.02gr/10ml – 0.001gr/10ml	1.35 ± 2.41	0.022*
0.008gr/10ml – 0.001gr/10ml	0.20 ± 1.10	0.428

Table 1 shows The cumulative effect of Benzoic acid on lifespan enhancement of *C. elegans* in different concentrations. The result was achieved with different concentrations of Benzoic acid showed negative results on lifespan when compared with control except for the 0.008gr/10ml concentrations of Benzoic acid, $p<0.05$ for all experiments

Figure 5: The Longevity Effect Different Concentrations of Benzoic Acid in *C. elegans*

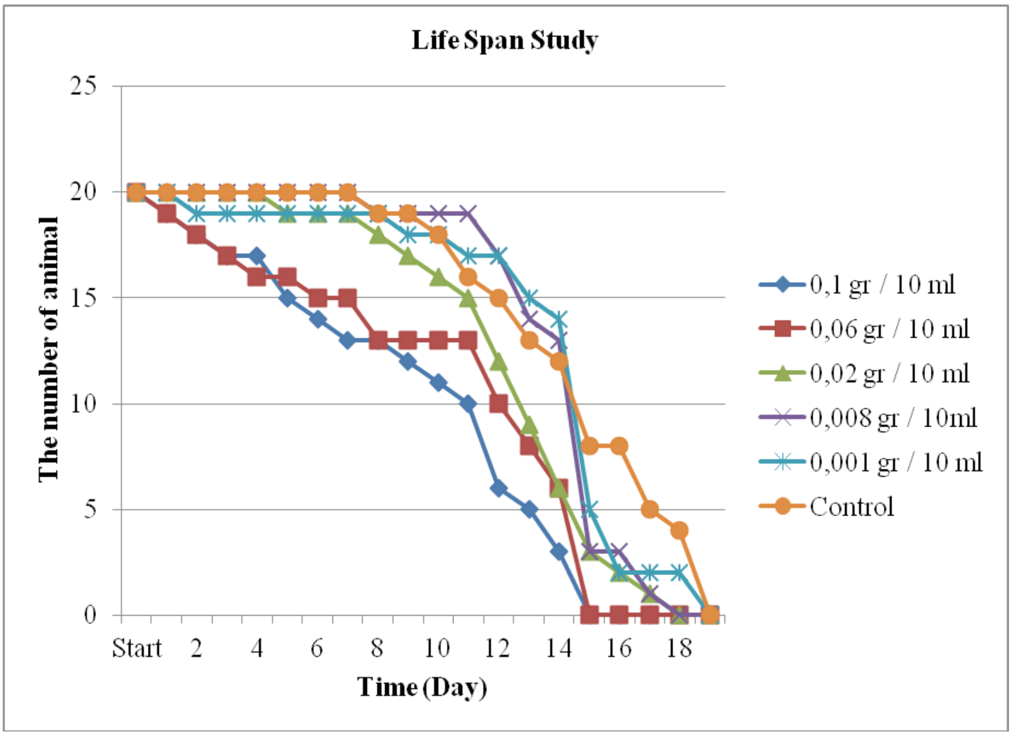

Figure 5 shows The longevity effect of Benzoic acid on lifespan enhancement of *C. elegans* in different concentrations of Benzoic

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