Influence of curcumin on lifespan if it is applied at the larval stage of *Drosophila melanogaster*

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Abstract. In this study we have applied the different doses of curcumin at the larval stages of a fruitfly *Drosophila melanogaster* and subsequently investigated its effect on the developmental duration and life expectancy of imago. It has been shown a 2-day delay in the hatching of the flies, which were developing in the medium containing 500 mM of curcumin. Exposure to curcumin significantly influenced on the average and maximum lifespan (ALS and MLS respectively) of all *Drosophila* in the study: ALS – $F=13.01$, $p<0.001$ for males and $F=14.3$, $p<0.001$ for females; MLS – $F=35.9$, $p<0.001$ for males and $F=16.7$, $p<0.001$ for females. Thus, the ALS in the males, which at the larval stage were kept in the medium containing 125 mM, 250 mM and 500 mM of curcumin, was significantly higher ($p<0.001$) comparing to the control. In females, such kind of significant increase in ALS has been shown at a dose of 500 mM of curcumin ($p<0.001$) in the medium during the developmental stage comparing to the control. In other words, the ALS of the imago has increased in correlation to the increase in the dose of curcumin applied at the developmental stage in males by 9%, 16%, 13%, and 23% and in females by 0%, 1%, 3%, 16% respectively. There has been also shown the sharp raise in MLS in both males and females, which at the larval stage were kept in the medium containing more than 125 mM of curcumin. To sum up we can assume that in this study consumption of curcumin at the larval stage of fruit flies significantly increased the developmental duration and life span of adult *Drosophila*, and this may demonstrate the effect of curcumin on the epigenetic programming of pace of life.

Keywords: Development; Life span; Curcumin; *Drosophila*

Many gerontologists believe that aging is a continuation of development, during which various parts of genome are turned on and off in a certain evolutionarily determined sequential order [1]. It can be assumed that when the program of development is being expanded, the work of the "clocks", which sets the speed of the aging program, is also being slowing down. Assuming the existence of a single program, it is possible to try to change the speed of its implementation with the help of various influences on the body during its developmental period. It is supposed that the main molecular mechanism of early programming processes is the specific point arrangement of epigenetic tags, which affect gene expression without changing DNA sequence [2]. The central mechanisms of epigenetic regulation of DNA expression is the methylation of cytosine residues in the regions of promoter genes. This normally leads to transcription blocking, and subsequently the post-translational modification (such as acetylation) of major histone proteins results into activation of transcription [3]. Investigation of substances affecting this kind of process is considered to be a very promising area of scientific research. Curcumin – a substance derived from turmeric is a typical plant phenolic compound with a numerous therapeutic properties [4]. There is evidence that curcumin can duplicate the effects of many epigenetic regulators, such as histone deacetylases, DNA methyl transferases, histone methyl transferases and histone demethylases [5, 6]. Curcumin treatment has been shown to promote hypomethylation of the promoter regions of tumor suppressor genes [7, 8, 9], thereby increasing

their expression in various cancer cell lines *in vitro*. The anti-inflammatory and antioxidant properties of curcumin also have been studied quite well [10]. The investigation of the molecular mechanisms of these properties demonstrated that curcumin can change the status of hypomethylation of TNF and other inflammatory genes and inhibit their expression [11]. Various preclinical and clinical studies show that curcumin has potential therapeutic effect against many chronic conditions, including neoplastic, neurological, cardiovascular, pulmonary, metabolic, and psychological diseases [12]. There is reason to believe that curcumin can slow down the aging process, as well as the onset or progression of many age-related diseases. The anti-aging potential of this polyphenolic compound has been repeatedly reported in literature [13]. The pleiotropic activity of curcumin is obviously associated with its ability to influence many signaling pathways, the study of which may increase the therapeutic capabilities of curcumin. Thereafter, the aim of the present work has been the investigation of the effect of different doses of curcumin applied in an immature *D. melanogaster* on its developmental duration and life span.

Material and methods

The study has been conducted on an outbred laboratory population of *Drosophila melanogaster*, line Oregon-R. Breeding and maintenance of the control group of flies was carried out under standard conditions: on a complete feed, which contained 4.0 g of sugar, 4.0 g of semolina, 1.0 g of agar-agar, and 1.0 ml of 20% benzoic acid solution in alcohol (to suppress mold growth) per 100 ml of water, at a normal temperature (25 ± 0.5 °C), in constant humidity and light/dark switching (12 to 12 hours per day). There were in total four experimental groups, which at the larval stage of development were kept in the nutrient medium containing 63, 125, 250, and 500 mmol/L of curcumin [Acros Organics, Belgium, <http://www.acros.com>]. Subsequently, all the adult insects were maintained under the normal conditions throughout the rest of their lives.

The fruitfly larvae were maintained in the 250 ml glass jars (5 pieces per each experimental group). In each jar we assessed the duration of development (DD – time passed from egg laying to hatching of 50% imago) and preimaginal survival (subgroup of flies, that went through the full development cycle from egg to imago). In order to determine the average lifespan (ALS) the adult flies were sorted by sex difference and placed separately into glass tubes (140 mm in height and 13 mm in diameter), which contained 2 ml of nutrient medium, at a population density of 25 insects each tube. There were 6 repeats per an experimental variant, thus, in each of the experimental groups, the ALS was determined in a total for 150 females and 150 males. The transferring to the fresh nutrient medium was carried out 3 times a week, and during this process the dead flies were counted. At the end of the experiment there was also determined the maximum lifespan – MLS – the lifespan of the last ten alive insects in each group. The significance of the effect of curcumin on the parameters of DD, ALS, and MLS was evaluated using univariate analysis of variance (ANOVA). The statistical evaluation of the differences between the control and each of the experimental groups was carried out using posterior comparisons (*Tukey's HSD post hoc tests*).

Statistical analysis. All data were analyzed using Statistics software version 7.0 (Statistics Inc., USA). One-way analysis of variance (ANOVA) with repeated measures followed by Tukey's HSD post hoc test was performed to determine group main effect. The level of statistical significance was set at $P < 0.05$. All data are presented as mean values $\pm$ SE within text, figures and table.

Results

The duration of development in *Drosophila*, which were kept in the nutrient medium containing 500 mM curcumin, increased by 2 days and was 11 days, that was significantly higher than usual 9 days exhibited in the control (Fig. 1). Thus in the control the developmental duration was 9 days, while the addition of curcumin to the nutrient medium at the larval stage extended this parameter up to 10-11 days. The curcumin doses less than 500 mM did not affect this feature.

Figure 1. The duration of development of *Drosophila*, which were kept in a nutrient medium containing different doses of curcumin.

There was revealed the direct dependence of ALS on duration of development (Fig. 2). For instance, the consumption of 500 mM curcumin at the developmental stage caused developmental delays for 2 days, and increase in ALS of *Drosophila* for 16-23%.

Figure 2. Dependence of average life span (ALS) on the duration of development (from egg to imago).

Note: * - $p < 0.05$ compared to the control (9 days)

The table presents the indicators of the ALS and MLS of imago, which at the larval stage have been kept in the nutrient medium containing different concentrations of curcumin. As it may be seen the significant effect of curcumin at the developmental stage on the ALS of *Drosophila* was revealed: $F = 13.01$, $p < 0.001$ for males; $F = 14.3$, $p < 0.001$ for females. Specifically, the ALS in the control was 59.31 ± 1.43 days in

males and 69.25 ± 1.35 days in females, but consumption of 125 mM, 250 mM, and 500 mM curcumin at the developmental stage significantly increased this parameter in males for 68.55 ± 1.40 days, 67.16 ± 1.49 days, and 73.15 ± 1.54 days, respectively, ($P < 0.001$) in comparison with control (Tab.).

Table

Average and maximum life span (ALS and MLS) of *D. melanogaster*, which have been maintained at the larval stage with different concentrations of curcumin

($M \pm m$, $N=150$)

Dose of curcumin, mM	Males		Females	
	ALS, days	MLS, days	ALS, days	MLS, days
0 (Control)	59.31 ± 1.43	83.33 ± 1.27	69.25 ± 1.35	90.67 ± 0.78
63	64.35 ± 1.23	88.60 ± 0.66	69.41 ± 1.19	89.80 ± 0.81
125	$68.55 \pm 1.40^{***}$	$93.60 \pm 0.41^{***}$	70.19 ± 1.43	$94.27 \pm 0.68^{**}$
250	$67.16 \pm 1.49^{***}$	$95.93 \pm 0.44^{***}$	71.49 ± 1.29	$93.60 \pm 0.72^*$
500	$73.15 \pm 1.54^{***}$	$98.00 \pm 0.44^{***}$	$80.50 \pm 0.95^{***}$	$96.67 \pm 0.27^{***}$

Note: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ comparing to the control (one-way analysis of variance ANOVA with subsequent Tukey's HSD test)

In females, a significant increase in ALS have been revealed only at a dose of 500 mM curcumin applied at the developmental stage: 80.50 ± 0.95 days, $P < 0.001$ compared to the control (Tab.). The overall effect of raising of curcumin dosage at the developmental stage (63 mM, 125 mM, 250 mM and 500 mM), increased the ALS of adult *Drosophila* comparing to the control by 9%, 16%, 13% and 23%, respectively, in males, but only for 0%, 1%, 3%, 16% in females.

With the use of one-way analysis of variance, it was revealed that consumption of curcumin at the developmental stage significantly increased the MLS of fruit flies: $F = 35.9$, $p < 0.001$ in males; $F = 16.7$, $p < 0.001$ in females. Specifically, this feature significantly increased in both sexes, starting from the dose of 125 mM curcumin and more applied at the developmental stage (Tab.). The adding of 125 mM, 250 mM, 500 mM of curcumin to the nutrient medium at the developmental stage led to the significant increase in the MLS of males: 93.60 ± 0.41 days, $P < 0.001$; 95.93 ± 0.44 days, $P < 0.001$; 98.00 ± 0.44 days, $P < 0.001$, respectively, in comparison to the control – 83.33 ± 1.27 days (Tab.). In females, the consumption of curcumin in doses of 125 mM, 250 mM, 500 mM at the developmental stage also led to a significant increase in ALS: 94.27 ± 0.68 days, $P < 0.01$; 93.60 ± 0.72 days, $P < 0.05$; 96.67 ± 0.27 days, $P < 0.001$, respectively, in comparison to the control – 90.67 ± 0.78 days (Tab.).

Figure 3. The survival curves of male and female *D. melanogaster*, which at the larval stage were maintained in the nutrient medium containing curcumin.

Discussion

Chronic oxidative stress resulting from a prooxidant/antioxidant imbalance between production of reactive oxygen species (ROS) and their elimination by antioxidant defense systems, as well as the associated with them systemic inflammation, are the processes that inevitably accompany and contribute to aging of cells [14, 15]. Damage of vital biomolecules including lipids, proteins, and nucleic acids, caused by ROS and other types of free radicals formed in a mitochondria during normal metabolic processes, are considered by many authors to be the main factors of aging [16]. It has been repeatedly demonstrated that ROS are involved in the main processes underlying aging, such as mitochondrial dysfunction [17], age-related depletion of telomeres [18, 19], autophagy [20], as well as depletion of stem cell populations [21]. It may be assumed that a decrease in oxidative stress will have a positive effect on vital functions and slow down the aging process. The antioxidant properties of curcumin have been confirmed in many studies [22]. It is known that curcumin eliminates free radicals of oxygen, inhibits lipid peroxidation, and protects cellular macromolecules such as DNA from oxidative stress [23]. It has been shown, that the addition of curcumin to the nutrient medium of young adult flies within 7 days increased the levels of superoxide dismutase and catalase along with a decrease in the activity of acetylcholinesterase (i.e. increased levels of acetylcholine along with a decrease in oxidative stress), which led to an increase in the life span [24]. The decrease in oxidative stress levels in response to curcumin consumption has been also observed in the study on the *Drosophila-PD* line (PD – Parkinson's disease), which is typically characterized by elevated levels of ROS [25]. In another study the addition of curcumin to the nutrition of imago increased the survival rate of *Drosophila* by increasing the thermal tolerance, which indicates that curcumin reduced the oxidative stress caused by heat shock [26]. The mechanism of the antioxidant properties of curcumin has been suggested: electron-donating groups, such as phenolic hydroxyl groups remove free radicals from cells [27]. Thus, the results obtained in our experiment can be explained by the antioxidant activity of curcumin. At the same time there is the information in the literature that suggests an alternative mechanism for increasing the duration of development and lifespan of *Drosophila*. Results similar to ours were demonstrated in a study in which curcumin was also added to the larval nutrition, and a significant increase in the average and maximum lifespan of adults was also observed. A similar effect was noticed when curcumin was added to the nutrition of young adults [28]. In that experiment, the gene expression of the larvae consuming curcumin was analyzed. The results obtained indicated that curcumin inhibited TOR (target of rapamycin) genes [28].

TOR activity is known to vary depending on the availability of nutrients. With their excess, the activity of the enzyme increases, prompting a cell to produce the proteins necessary for their assimilation and division. In the opposite case, TOR decreases activity, protein production stops, and the cell stores nutrients. At the same time, the process of autophagy begins – the destruction of defective structures (proteins with incorrect conformation, non-functioning mitochondria, etc.) with the formation of by-products, which can be used as nutrients or building materials. Thus, a natural decrease in TOR activity occurs with fasting, that may explain the beneficial effect of a calorie restricted diet (CRD) on vitality and lifespan parameters [29]. The TOR system also responds to other stress factors, including a decrease in oxygen levels and DNA damage.

To summarize, we can say that in all cases when a stressful situation arises, TOR activity decreases. As a result, fewer proteins are produced, and a cell can spend resources on DNA repair and other urgent needs, but at the same time the division processes slow down, that is, development slows down, which is demonstrated by the results of our work: curcumin, inhibiting the TOR pathway, significantly slows down the development of larvae. These data support the hypothesis that curcumin is a mimetic of CRD. It is known that TOR inhibition increases the lifespan either in yeasts or mammals, that demonstrates the universality and evolutionary stability of this mechanism. How are ROS levels and TOR pathway activity connected? It is known that ROS, along with other factors (such as excess food, growth factors), activate TOR, thereby accelerating aging. Thus, curcumin, by binding ROS, blocks one of the pathways of TOR activation, which leads to the effects which are typical for TOR inhibition [30].

The results of our study indicate that the putative mechanism of TOR inhibition is "remembered" and continues to work even after the metamorphosis of *Drosophila* and in the absence of curcumin in the nutrition. Therefore, it may be supposed that curcumin is an epigenetic programming agent.

Conclusions

In this study we have used different doses of curcumin in order to slow the rate of development. It has been found that the addition of curcumin at the developmental stage significantly prolongs this period, as well as and lifespan in *Drosophila* ($p < 0.001$). With an increase in the dose of curcumin added at the developmental stage (63 mM, 125 mM, 250 mM, and 500 mM) the life expectancy of adult fruit flies increases in comparison to the control by 9%, 16%, 13%, and 23% respectively in males and by 0%, 1%, 3%, 16% in females ($p < 0.001$). It may be assumed that curcumin influences on the epigenetic programming of the pace of life.

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