Halim, J. E. K., et al. International Journal of Biomedical Science and Travel Medicine (IJBSTM)
Volume 1, Number 1, 2024
https://doi.org/10.22225/ijbstm.1.1.2024.24-29

International Journal of Biomedical Science and Travel Medicine (IJBSTM)

Original Article **Open Access**

Evaluating Curcumin Intake on Metabolism-Related Genes in *Drosophila melanogaster*

Jonathan Elbert Karsten Halim[1], Asbah Asbah[1], Nadila Pratiwi Latada[2], Mukarram Mudjahid[3], Usmar Usmar[3], Risfah Yulianti[4], and Firzan Nainu[2,3*]

Abstract

Background Aging entails a gradual deterioration of physiological functions within the body. Current research provides evidence suggesting that curcumin may extend the lifespan of fruit flies by mitigating the effects of aging. However, the precise concentration of curcumin necessary to induce favorable phenotypic and molecular outcomes in fruit flies has yet to be determined.

Methods The study utilized the capillary feeder (CAFE) assay on male Oregon-R flies, and examined the expression of the srl and pepck genes through the reverse transcriptase quantitative PCR (RT-qPCR) method.

Results An elevation of curcumin consumption was examined in the treatment groups that were provided with feed containing curcumin concentrations of 50 µM and 250 µM. This observation is consistent with the increased lifespan noted in the Drosophila groups consuming higher concentrations of curcumin. Meanwhile, molecular analysis at the expression level of the srl and pepck genes revealed no significant change in gene expression between the treatment and control groups.

Conclusion The use of the CAFE assay assists researchers in quantitatively measuring the amount of curcumin intake in Drosophila melanogaster. However, the consumption of curcumin in this experiment did not demonstrate a significant impact on the metabolism-related genes of Drosophila, srl and pepck genes.

Keywords: Aging; curcumin intake; fruit fly; RT-qPCR; srl; pepck

Correspondence: firzannainu@unhas.ac.id

1. Undergraduate Program in Pharmacy, Faculty of Pharmacy, Hasanuddin University, Tamalanrea, Makassar 90245, Indonesia

2. Unhas Fly Research Group, Faculty of Pharmacy, Hasanuddin University, Tamalanrea, Makassar 90245, Indonesia

3. Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Tamalanrea, Makassar 90245, Indonesia

4. Department of Pharmaceutical Science and Technology, Faculty of Pharmacy, Hasanuddin University, Tamalanrea, Makassar 90245, Indonesia

Introduction

Aging is a process characterized by a decline in bodily functions (Morrison & Newell, 2012). With advancing age, the risk of both infectious and degenerative diseases increases (Guo et al., 2022). Aging is often associated with a decline in mitochondrial function, leading to reduced ATP production, enzyme activity, and protein production in the mitochondrial matrix (Srivastava, 2017; Klaips et al., 2018; Yu & Hyun, 2021). Due to the intricate dynamics of aging, it serves as the fundamental focus for research in this area, with the objective of addressing and managing diseases linked to aging (Zhang et al., 2023). One compound that has been identified for its anti-aging properties is curcumin (Bielak-Zmijewska et al., 2019; Bahrami et al., 2021).

Previous findings demonstrate that curcumin has the capacity to mitigate the generation of reactive oxygen species (ROS), thereby enhancing cognitive function restoration in rats (Sun et al., 2015). Another study revealed that curcumin can extend the lifespan of *Drosophila melanogaster* by providing protection against oxidative stress and enhancing locomotor activity in *Drosophila* (Lee et al., 2010). In addition, curcumin exerts its anti-aging effect by increasing the expression levels of genes related to *D. melanogaster* metabolism, including *srl* and *pepck* (Asfa et al., 2023).

The Spargel hormone-coding gene (*srl*) and phosphoenolpyruvate carboxykinase gene (*pepck*) are associated with the aging process (Ng et al., 2017; Onken et al., 2020). The *srl* gene in *D.*

melanogaster is homologous to *PGC-1α* in humans (Tinkerhess et al., 2012), while the pepck gene in *Drosophila* is homologous to the *PCK2* gene in humans (Onken et al., 2020). Increased expression of the *srl* gene leads to increased oxygen consumption in mitochondria, resulting in elevated ATP production and protein production in the mitochondrial matrix (Mukherjee & Duttaroy, 2013). Meanwhile, an increase in the expression of *pepck* gene causes enhanced regulation and energy metabolism, potentially extending the lifespan of model organisms (Chatterjee & Perrimon, 2021).

Drosophila melanogaster is one of the model organisms used in aging research. The advantages of using *D. melanogaster* as a model organism include easy care, a relatively short lifespan, and a 75% homology with human genes (Tsurumi & Li, 2020). Curcumin has been proven to influence the expression of *pepck* and *srl* genes in *melanogaster*. However, as of now, there is no study on the quantity of curcumin (curcumin intake) consumed by flies to achieve these effects.

Curcumin intake can be measured using the capillary feeder (CAFE) assay. The CAFE assay is a method used to measure the food intake of *D. melanogaster* provided in glass capillary tubes placed in vials (Diegelmann et al., 2017). According to the previous study, the CAFE assay is a method to understand the physiology and regulation of appetite, as well as to monitor oral drug administration (Ja et al., 2007). This study aims to determine the curcumin intake of *D. melanogaster* associated to its impact on the expression level of *srl* and *pepck* genes.

Methods

Fly Stock. This study utilized male flies of the *Oregon R* genotype obtained from the Laboratory Host Defense and Responses at Kanazawa University. The flies were bred and cultivated in culture vials containing food. The age range of the flies employed in the experiment was 4 -7 days, and were maintained at 25°C.

Sample Preparation. Curcumin samples were prepared by initially creating a stock solution with a concentration of 50 mM. Following this, dilution was carried out using 96% ethanol to obtain concentrations of 10 mM and 2 mM. Subsequently, each experimental solution was mixed into the fly food with final curcumin concentrations of 10 µM, 50 µM, and 250 µM.

Capillary Feeder (CAFE) Assay. We utilized the CAFE Assay method according to the established protocol (Diegelmann et al., 2017), with a slight modification. After the filter paper was placed at the bottom of the vial as a humidity indicator, subsequently, 3 male flies were transferred into the vial, and sealed using plugs. A hole was then created in the middle of the vial plugs, and a microtips was inserted into the prepared hole. The capillary tube containing the food was entered into all vials at the same height, approximately 3-4 cm from the vial's top (**Fig. 1**). The capillary tubes were marked with a marker to ensure identification during changes in food volume (initial marking). As the filter paper dried during the testing period over several days, clean water was applied every 24 hours and dropped through the Drosophila plug to maintain constant humidity during the experiment. Capillary tubes were replaced every 24 hours, and the lower meniscus of the capillary containing the food (final marking) was marked with a marker in an upright position. The intake volume can be measured using the following formula:

$$IV = [\frac{1}{4}\pi d^2 \, x \, h]_{sample} - [\frac{1}{4}\pi d^2 \, x \, h]_{vapor\ control}$$

Note:

IV : Intake volume (µl)

d : Diameter (mm)

h : The difference between the lower limit and the upper limit (mm)

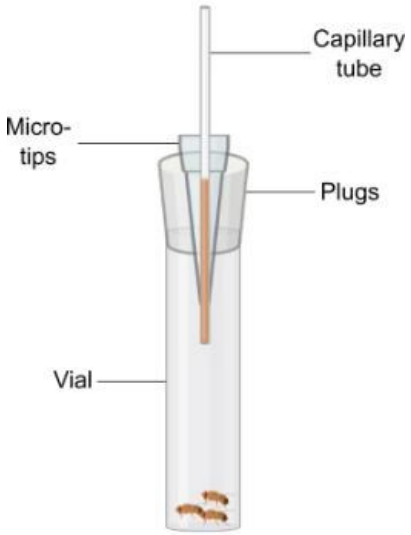

Figure 1. CAFE assay design with a slight modification.

Survival Assay. Survival assay was carried out to assess the effect of curcumin administration through the CAFE assay on the lifespan of *Oregon R*. Daily observations were carried out for a period of 14 days, by recording the number of flies that experienced mortality after treatment.

Gene Expression Analysis. RNA was isolated from five flies taken from each treatment group and transferred into Treff tubes. Extraction was carried out using the SV Total RNA Isolation System (Promega) according to the manufacturer's protocol. The expression levels of *srl* and *pepck* genes were quantitatively assessed through the reverse transcriptase quantitative PCR (RT- qPCR) method. Separate analyses were conducted using

forward and reverse (one set) of *srl* and *pepck* primers in a 10 µl reaction volume, employing the GoTaq 1-Step RT-qPCR System (Promega) as the manufacturer's instructions. Following a similar RT-qPCR protocol, the RNA level of the host ribosomal protein *rp49* (utilized as an internal control) was investigated using one set of *rp49* primers. using RotorGene Q thermal cycler (Qiagen, Germany) PCR with the following temperature (1) cDNA synthesis: 37°C, 15 mins,

PCR cycles: 95°C,10 mins, (3) Denaturation: 95°C,10 second, (4) Annealing: 60°C, 30 secs, (5) Extension: 72°C,1 0 secs, in 40 cycles.

Table 1. Primer sequences used in RT-qPCR

Genes	Primer Sequences	
	Forward	Reverse
srl	5'–CTCTTGGAGTCCGAGATCCGCAA–3'	5'-GGGACCGCGAGCTGATGGTT–3'
pepck	5'–CCGCCGAGAACCTTATTGTG–3'	5'–AGAATCAACATGTGCTCGGC–3'
rp49	5'–GACGCTTCAAGGGACAGTATCTG–3'	5'–AAACGCGGTTCTGCATGAG–3'

Data Processing and Analysis. Data obtained from the survival test will be analyzed using the LogRank method. Meanwhile, data from the reproduction and gene expression assays will be analyzed using the One-Way ANOVA method (post hoc Tukey) with GraphPad Prism® 9.

Results and Discussion

In this study, we first quantified the volume of food intake in male flies through the CAFE assay. This assessment aimed to measure the food volume intake (contains compounds) while concurrently evaluating the effectiveness of the CAFE assay method as one of an approach for administering compounds to *Drosophila*. The results obtained are presented in **Table 2**.

Table 2. Food Intake (µl) during treatment.

Day	Intake Volume (µl)				
	UC	SC	C10 µM	C50 µM	C250 µM
4	0,84	0,74	0,96	0,83	1,15
5	0,34	0,66	0,47	0,43	0,46
6	0,39	0,71	0,59	0,94	0,65
7	2,88	2,45	3,28	3,99	3,15
8	0,91	1,32	1,14	1,31	1,27
9	0,51	0,63	0,50	0,93	0,80
10	1,24	0,7	0,88	0,89	1,10
11	1,20	1,35	1,25	1,59	1,35
12	0,62	0,83	0,82	1,05	0,93
13	1,25	2,11	2,00	2,42	2,00
14	0,64	0,53	0,15	0,73	0,87

UC: Untreated Control; SC: Solvent Control; C: Curcumin

The first to the third days are considered as the adaptation period for Drosophila to the CAFE assay conditions, hence the calculation of intake volume was performed on the subsequent day. Based on the data presented in **Table 2**, it can be observed that the significant and highest food consumption occurred on the 7th day of the treatment. Furthermore, utilizing the calculated volume results, we determined the quantity of curcumin consumed by Drosophila, as detailed in **Table 3**.

Table 3. Measurement of curcumin intake during treatment (14 days)

Day	Curcumin Intake (µg)		
	10 µM	50 µM	250 µM
4	0,0035	0,0153	0,1059
5	0,0017	0,0079	0,0424
6	0,0022	0,0173	0,0599
7	0,0121	0,0735	0,2901
8	0,0042	0,0241	0,1170
9	0,0018	0,0171	0,0737
10	0,0032	0,0164	0,1013
11	0,0046	0,0293	0,1243
12	0,0030	0,0193	0,0856
13	0,0074	0,0446	0,1842
14	0,0006	0,0134	0,0801

Aligned with the increasing amount of feed consumed by flies on the 7th day, there was a corresponding rise in curcumin intake on that day, specifically at levels of 0.0121 µM, 0.0735 µM, and 0.2901 µM in the treatment groups provided with curcumin (10 µM, 50 µM, and 250 µM). These findings indicate a concentration-dependent increase in curcumin consumption. We hypothesize that flies receiving feed through a capillary feeder exhibit diminished feed intake, and thus, the presence of high curcumin concentrations may aid flies in ATP production for survival in such conditions. Based on this hypothesis, we conducted observations on the survival of flies fed with curcumin-containing feed through a capillary feeder.

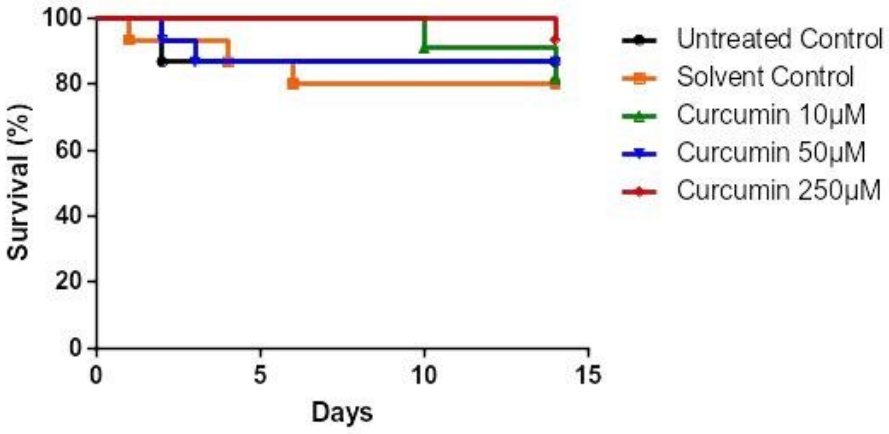

Figure 2. Curcumin consumption extends the lifespan of male flies compared to the control groups.

According to the graph (**Fig. 2**) it is apparent that flies consuming high concentrations of curcumin through a capillary feeder exhibit a prolonged lifespan compared to the control group that did not receive curcumin. Subsequently, we conducted a molecular analysis to elucidate the molecular correlations underlying the observed increase in Drosophila survival in the curcumin-treated group with genes associated with metabolism in Drosophila, employing the RT-qPCR method.

The *srl* gene encodes the Spargel protein, a crucial component in energy production and mitochondrial biosynthesis of Drosophila (Mukherjee & Duttaroy, 2013). On the other hand,

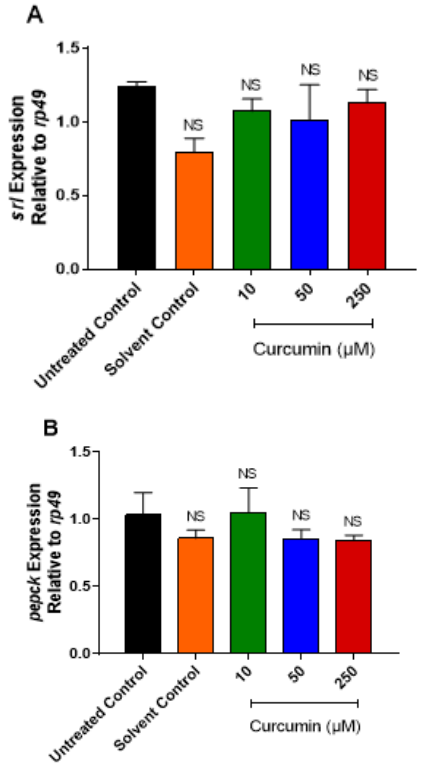

Figure 3. Curcumin consumption had no significant effect on the expression level of both *srl* (A) and *pepck* (B) genes. NS: non-significant.

the *pepck* gene is linked to gluconeogenesis, which directly affect the lifespan of Drosophila (Onken et al., 2020). Based on the molecular analysis results (**Fig. 3**) indicate that the intake of curcumin does not significantly affect the expression levels of genes related to metabolism in Drosophila, specifically *srl* and *pepck*. The relatively brief 14-day duration of curcumin treatment may be a contributing factor, as it fails to manifest a significant effect at the molecular level.

Conclusion

The utilization of the capillary feeder method presents a beneficial tool for researchers investigating the concentration of ingested compounds in model organisms, particularly *D. melanogaster*. Despite of its advantages, it is worth noting that the method, given the limited food quantity in the capillary tube, necessitates an extended exposure duration to effectively capture a comprehensive understanding of both phenotypic and molecular effects. Moreover, this study suggests that employing the modification of compound administration routes in Drosophila by the capillary feeder method offers a quantitative, convenient, and cost-effective depiction, and could potentially be widely applicable in developing countries.

Reference

1. Asfa, N., Widianto, A. S., Pratama, M. K. A., Rosa, R. A., Mu'arif, A., Yulianty, R., & Nainu, F. (2023). Curcumin-mediated gene expression changes in Drosophila melanogaster. *Pharmacy Education*, *23* (2), 84–91. https://doi.org/10.46542/pe.2023.232.8491

2. Bahrami, A., Montecucco, F., Carbone, F., & Sahebkar, A. (2021). Effects of Curcumin on Aging: Molecular Mechanisms and Experimental Evidence. *BioMed Research International*, *2021*. https://doi.org/10.1155/2021/8972074

3. Bielak-Zmijewska, A., Grabowska, W., Ciolko, A., Bojko, A., Mosieniak, G., Bijoch, Ł., & Sikora, E. (2019). The role of curcumin in the modulation of ageing. *International Journal of Molecular Sciences*, *20*(5). https://doi.org/10.3390/ijms20051239

4. Chatterjee, N., & Perrimon, N. (2021). What fuels the fly: Energy metabolism in Drosophila and its application to the study of obesity and diabetes. *Science Advances*, *7*(24), 1–14. https://doi.org/10.1126/sciadv.abg4336

5. Diegelmann, S., Jansen, A., Jois, S., Kastenholz, K., Escarcena, L. V., Strudthoff, N., & Scholz, H. (2017). The CApillary feeder assay measures food intake in *Drosophila melanogaster*. *Journal of Visualized Experiments*, *2017*(121), 1–14. https://doi.org/10.3791/55024

6. Guo, J., Huang, X., Dou, L., Yan, M., Shen, T., Tang, W., & Li, J. (2022). Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. *Signal Transduction and Targeted Therapy*, *7*(1). https://doi.org/10.1038/s41392-022-01251-0

7. Ja, W. W., Carvalho, G. B., Mak, E. M., De La Rosa, N. N., Fang, A. Y., Liong, J. C., Benzer, S. (2007). Prandiology of Drosophila and the CAFE assay. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(20), 8253–8256. https://doi.org/10.1073/pnas.0702726104

8. Klaips, C. L., Jayaraj, G. G., & Hartl, F. U. (2018). Pathways of cellular proteostasis in aging and disease. *Journal of Cell Biology*, *217*(1), 51–63. https://doi.org/10.1083/jcb.201709072

9. Lee, K. S., Lee, B. S., Semnani, S., Avanesian, A., Um, C. Y., Jeon, H. J., Jafari, M. (2010). Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in *Drosophila melanogaster*. *Rejuvenation Research*, *13*(5), 561–570. https://doi.org/10.1089/rej.2010.1031

10. Morrison, S., & Newell, K. M. (2012). Aging, neuromuscular decline, and the change in physiological and behavioral complexity of upper-limb movement dynamics. *Journal of Aging Research*, *2012*. https://doi.org/10.1155/2012/891218

11. Mukherjee, S., & Duttaroy, A. (2013). Spargel/dPGC-1 is a new downstream effector in the insulin-TOR signaling pathway in Drosophila. *Genetics*, *195*(2), 433–441. https://doi.org/10.1534/genetics.113.154583

12. Ng, C. H., Basil, A. H., Hang, L., Tan, R., Goh, K. L., O'Neill, S., … Lim, K. L. (2017). Genetic or pharmacological activation of the Drosophila PGC-1α ortholog spargel rescues the disease phenotypes of genetic models of Parkinson's disease. *Neurobiology of Aging*, *55*, 33–37. https://doi.org/10.1016/j.neurobiolaging.2017.03.017

13. Onken, B., Kalinava, N., & Driscoll, M. (2020). *Gluconeogenesis and PEPCK are critical components of healthy aging and dietary restriction life extension*. *PLoS Genetics* (Vol. 16). https://doi.org/10.1371/JOURNAL.PGEN.1008982

14. Srivastava, S. (2017). The mitochondrial basis of aging and age-related disorders. *Genes*, *8*(12). https://doi.org/10.3390/genes8120398

15. Sun, Y., Hu, X., Hu, G., Xu, C., & Jiang, H. (2015). Curcumin attenuates hydrogen peroxide-induced premature senescence via the activation of SIRT1 in human umbilical vein endothelial cells. *Biological and Pharmaceutical Bulletin*, *38*(8), 1134–1141. https://doi.org/10.1248/bpb.b15-00012

16. Tinkerhess, M. J., Healy, L., Morgan, M., Sujkowski, A., Matthys, E., Zheng, L., & Wessells, R. J. (2012). The Drosophila PGC-1α homolog spargel modulates the physiological effects of endurance exercise. *PLoS ONE*, *7*(2). https://doi.org/10.1371/journal.pone.0031633

17. Tsurumi, A., & Li, W. X. (2020). *Aging mechanisms—A perspective mostly from Drosophila* . *Advanced Genetics* (Vol. 1). https://doi.org/10.1002/ggn2.10026

18. Yu, G., & Hyun, S. (2021). Proteostasis-associated aging: lessons from a Drosophila model. *Genes and Genomics*, *43*(1). https://doi.org/10.1007/s13258-020-01012-9

19. Zhang, Y., Li, Q., Niu, Y., Wei, K., Wang, X., Niu, B., & Zhang, L. (2023). Research progress on aging mechanism and drugs and the role of stem cells in anti-aging process. *Experimental Gerontology*, *179* (June), 112248. https://doi.org/10.1016/j.exger.2023.112248