Research Article **Open Access**

Regetting-18 complex powder (R-18CP) - A formulation of components from food-medicine and its anti-aging effects

Lan YUAN[1], Paul F. DAVIS[1]*

Trinity Bioactives Ltd, 69 Gracefield Road, Gracefield, Lower Hutt, New Zealand

*Corresponding author: Paul F. Davis, Trinity Bioactives Ltd, 69 Gracefield Road, Gracefield, Lower Hutt, New Zealand.

Submission Date: May 20th, 2025; **Acceptance Date:** June 29th, 2025; **Publication Date:** July 8th, 2025

Please cite this article as: Yuan L., Davis P. F. Regetting-18 complex powder (R-18CP) - A formulation of components from food-medicine and its anti-aging effects. *Functional Foods in Health and Disease* 2025; 15(7): 369 – 379.
DOI: https://doi.org/10.31989/ffhd.v15i7.1647

ABSTRACT

Background: Aging is a natural process that affects all living beings. This process leads to various physiological and pathological changes. Human health has traditionally relied on a combination of dietary supplements and herbal remedies (food-medicine homogeneity) to combat the effects of aging. Recent scientific advancements have provided more profound insights into the mechanisms by which these traditional substances contribute to the anti-aging impacts through their active components.

Objectives: A formulation called Regetting-18 Complex Powder (R-18CP) was investigated using multiple aging markers that were hypothesized to inhibit cellular senescence. This formulation included several bioactive ingredients known for their anti-aging properties, such as *Panax ginseng, Ganoderma lucidum,* and Polygonati rhizome.

Methods: Adult male and female zebrafish were paired overnight, and embryos were collected the following day. Six experimental groups were established, each consisting of 10 fish, and all groups were conducted in triplicate. The groups included: (1) Control, (2) Model, (3) Positive control (treated with 5 μg/mL resveratrol), and (4) three R-18CP treatment groups (treated with 1 μg/mL, 0.5 μg/mL, and 0.1 μg/mL, respectively). At 72 hours post-fertilization, various assays were performed: 10 fish per group were stained for β-galactosidase, another 10 for reactive oxygen species (ROS), 10 were analyzed for mitochondrial membrane potential, 50 were used to assess telomerase levels, and DNA was extracted from 30 fish for telomere length analysis.

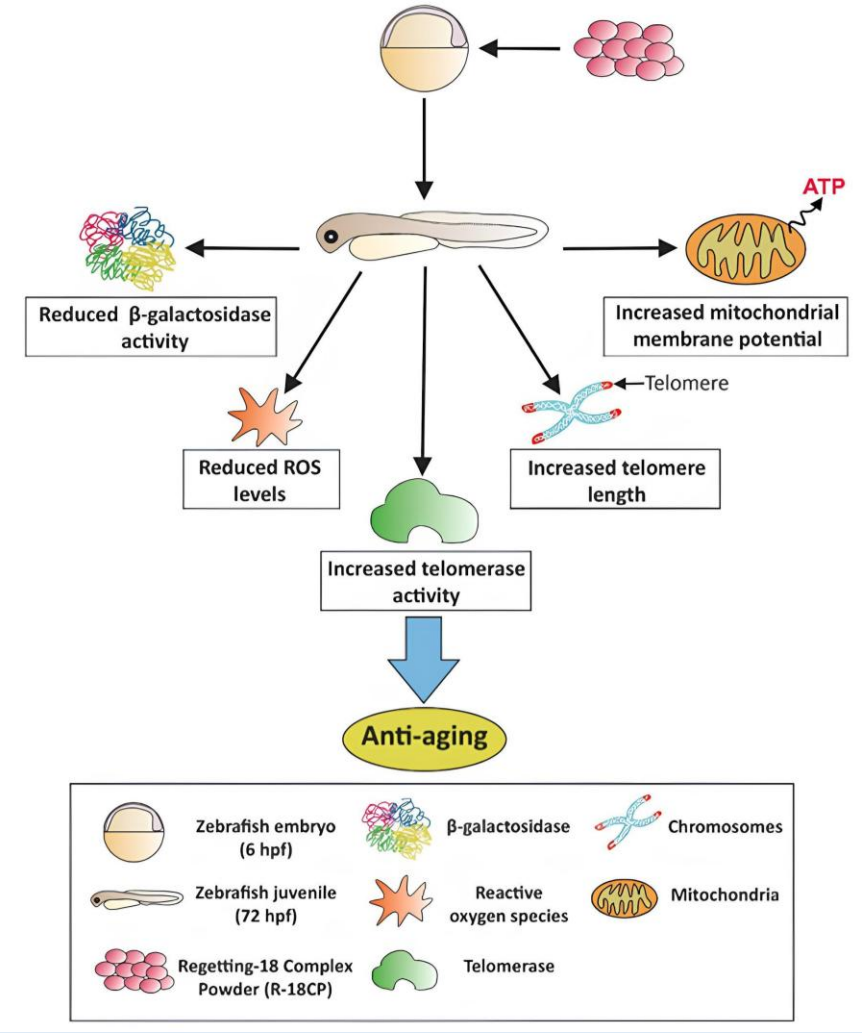
Results: R-18CP reduced β-galactosidase staining intensity in a dose-dependent manner. Increasing concentrations of R-18CP were associated with decreased ROS staining intensity, elevated telomerase levels, and longer telomeres.

Additionally, the mitochondrial membrane potential staining intensity increased in proportion to the R-18CP concentration.

Conclusion: Based on the five distinct aging markers assessed in the zebrafish model, R-18CP may be able to restrict aging. Further studies using human models are recommended and necessary.

Novelty of the Study: This study is the first to report that an R-18CP can exert anti-aging effects through telomere protection and improved mitochondrial function in zebrafish models. This suggests R-18CP is a potential therapeutic compound for anti-aging.

Key words: anti-aging, food-medicine homogeneity, zebrafish, β-galactosidase, telomere, telomerase, reactive oxygen, membrane potential



Graphical Abstract: Regetting-18 complex powder (R-18CP) - A formulation of components from food-medicine and its anti-aging effects

INTRODUCTION

Aging is a process in which a functional and organic decline occurs in the body, involving various levels of damage to tissues, organs, genes, proteins, cells, and intercellular communication systems. Specific manifestations include telomere shortening, accumulation of DNA damage, abnormal activation of oncogenes, metabolic changes, and excessive production of ROS. Telomeres, as nuclear protein structures covering the ends of each chromosome arm, are susceptible to age-related and oxidative stress-induced damage [1, 2]. As telomeres shorten due to cell division, apoptosis, and death of cells accelerate within the body, ultimately leading to increased aging. The production of telomerase can slow down the shortening of telomeres, thereby delaying the aging process [3]. When proteins are damaged, the balance of protein synthesis, folding, and degradation within the body becomes disrupted, which accelerates aging and the development of age-related diseases [4]. At the cellular level, there is an increase in the β-galactosidase enzyme. This leads to damaged stem cells, mitochondrial dysfunction, and nutritional imbalance. Damage to stem cells leads to a decline in tissue repair function, resulting in irreversible aging. Meanwhile, mitochondrial dysfunction and nutritional imbalance accelerate this process [5].

The aging process in zebrafish shares strong similarities with human aging, both characterized by the accumulation of lipofuscin and cognitive decline [6, 7]. Studies have shown that drug-induced aging models in zebrafish exhibit similar damage to humans in terms of DNA, cellular tissues, and organs [8]. When H2O2 enters the body of a zebrafish, it generates a large number of free radicals, which induce oxidative stress responses when in excess. This causes lipid peroxidation, which destabilizes biological membranes, produces lipofuscin, and ultimately damages nuclear and mitochondrial DNA. This leads to protein oxidation damage and macromolecular cross-linking, thereby inducing aging in zebrafish and shortening their lifespan [9].

The concept of "food-medicine homology" refers to substances that have been used in traditional medicine and are found naturally in foods [10]. These substances often exhibit beneficial effects beyond their primary uses, making them potential candidates for modern medical research [10]. R-18CP is a complex of food-medicine homogeny mainly from *panax ginseng*, *ganoderma lucidum*, polygonati rhizoma. The active components responsible for the anti-aging effects observed in those food-medicine homology are typically polyphenols, saponins, glycosides, and other bioactive compounds [10-12]. In this research, R-18CP has been studied for its anti-aging effects in a zebrafish experimental system by utilizing aging biomarkers such as ROS, the enzymes β-galactosidase and telomerase, telomeres, and mitochondrial changes. Resveratrol, a polyphenolic compound found in red wine and grapes, has been extensively studied for its anti-aging properties. Research indicates that this compound protects cells from oxidative damage and extends their lifespan [13]. This characteristic allowed for its use as a positive control in this study.

MATERIALS AND METHODS

- **R-18CP**, the test compound, was provided by **Suka Health Industry** (Shenzhen, China).
- **Zebrafish** were bred and housed by **Baihuan Biotechnology** (Guangzhou, China).
- **Resveratrol** (3,4',5-Trihydroxystilbene) was purchased from **Thermo Fisher Scientific** (Fair Lawn, NJ, USA).
- **60× E3 solution** was prepared according to the *Cold Spring Harbor Protocols* (2011) using the following components:
- 34.8 g **sodium chloride (NaCl)**
- 1.6 g **potassium chloride (KCl)**
- 5.8 g **calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)**
- 9.78 g **magnesium chloride hexahydrate**

($MgCl_2 \cdot 6H_2O$)

These were dissolved in deionized water to a total volume of 2 L. The pH was adjusted to 7.2 using **sodium hydroxide (NaOH)**, and the solution was autoclaved.

To prepare **1× E3 medium**, 16.5 mL of the 60× stock was diluted to 1 L with deionized water, and 100 µL of 1% **methylene blue** (Sigma-Aldrich, St. Louis, MO, USA) was added.

- **Galactose** (Sigma-Aldrich, St. Louis, MO, USA)
- **Phosphate-buffered saline (PBS)** (Sangon Biotech, Shanghai, China)
- **β-galactosidase staining kit** (Suzhou Lanjieke Biotechnology, Suzhou, China)
- **2′,7′-Dichlorofluorescin diacetate (DCFH-DA)** (Solarbio, Beijing, China)
- **Tricaine mesylate (MS-222)** (Sigma-Aldrich, St. Louis, MO, USA)
- **Telomerase ELISA kit** (Hufeng Chemical, Shanghai, China)
- **DNA extraction kit** (Zhuangmeng Biotechnology, Tianjin, China)
- **Nicotinamide adenine dinucleotide (NAD^+) probe kit** (Beyotime Biotechnology, Shanghai, China)

Methods

Animal rearing and processing: The zebrafish experiments were approved by the Institutional Animal Care and Use Committee (MDL2025-03-04-03). Adult zebrafish were housed in a recirculating water system (Hunter Biotech, Hangzhou, China) at 28 °C under a 14-hour light/10-hour dark cycle and were fed three times daily. To obtain embryos, male and female zebrafish were paired at a 1:1 ratio in the evening and separated by a barrier. The barrier was removed the following morning, allowing egg laying within the first hour of the light cycle. Collected embryos were transferred to 10-cm Petri dishes containing 1× E3 solution supplemented with 0.3 ppm methylene blue and incubated in an artificial climate incubator (BluePard, Yiheng Scientific Instrument, Shanghai, China) at 28.5 °C under the same light/dark cycle until treatment

Determination of maximum tolerated concentration (MTC) of R-18CP: Experimental groups included a control group and five R-18CP treatment groups at different concentrations (0.1 µg/mL, 0.5 µg/mL, 1 µg/mL, 5 µg/mL, and 25 µg/mL). Zebrafish embryos at 6 hours post-fertilization (6 hpf) were randomly selected and exposed to the respective R-18CP concentrations in 6-well plates (5 mL per well), with 10 embryos per well and three replicates per group. The media were refreshed daily until 72 hpf. Dead embryos were promptly removed and recorded. At 72 hpf, the mortality rate was assessed in each well, and the MTC was determined. Additionally, 10 embryos were randomly selected from each group to measure heart rate, calculated by counting heartbeats over a 20-second period. Morphological abnormalities were also assessed at 72 hpf. These malformations were recorded and photographed to calculate the malformation rate. Specific defects, including pericardial edema, yolk sac cysts, abnormal bleeding in the heart or brain, body curvature, shortened jaw or body length, and absence of a swim bladder, were documented and classified according to their type.

Groups and treatment: Six experimental groups were established: (1) Control group, (2) Model group, (3) Positive control group (treated with 5 µg/mL resveratrol), and (4) three R-18CP treatment groups (administered at 1 µg/mL, 0.5 µg/mL, and 0.1 µg/mL, respectively). At 6 hours post-fertilization (hpf), zebrafish embryos were randomly assigned to the six experimental groups to minimize selection bias. Embryos were then transferred to 6-well plates, with 10 embryos per well and three replicates per group. The control group was maintained

in a standard E3 solution, while the model group was exposed to an E3 solution supplemented with 40 mg/mL D-galactose to induce aging-like conditions [14]. The positive control group received 5 μg/mL resveratrol in addition to the model treatment. Similarly, the R-18CP treatment groups received their respective concentrations of R-18CP in addition to the D-galactose treatment.

β-galactosidase staining intensity test for anti-aging effects of samples: β-galactosidase staining was performed to assess the anti-aging effects of the samples. Following the treatment protocol described in Section 2.2, the culture medium was changed daily. At 72 hpf, 10 zebrafish from each group were randomly selected and transferred to 1.5 mL Eppendorf tubes. The fish were washed three times with 1× PBS for 5 minutes each and then fixed in 4% paraformaldehyde (PFA) at 4°C for 24 hours. After fixation, the PFA was removed, and 500 μL of β-galactosidase staining working solution—prepared according to the kit manufacturer's instructions—was added to each tube. The tubes were incubated overnight at 37 °C in the dark. Following incubation, the staining solution was discarded, the fish were rinsed with PBS, and then imaged. The staining intensity was subsequently quantified using ImageJ software.

Detection of ROS level: ROS levels were measured to evaluate the anti-aging effects of the samples. Following the grouping and treatment protocol described in Section 2.2, 10 zebrafish from each group were randomly selected at 72 hpf and incubated with the DCFH-DA probe in the dark for 1 hour (according to the supplier's instructions). After incubation, the fish were administered anesthesia with 0.01% MS-222 to capture fluorescence images. Fluorescence intensity, indicative of ROS levels, was quantified using ImageJ software.

Detection of Telomerase Activity: Telomerase activity was measured to assess the anti-aging effects of the samples. Following the grouping and treatment protocol described in Section 2.2, 50 zebrafish from each group were homogenized at 72 hpf. Briefly, physiological saline was added to a 1.5 mL microcentrifuge tube containing juvenile fish at a 1:9 (mass: volume) ratio. The mixture was homogenized on ice and centrifuged at 5000 × g for 10 minutes at 4°C. Telomerase activity was then quantified using an ELISA kit (Shanghai Hufeng Chemical Co., Ltd.), according to the manufacturer's instructions. The results were statistically analyzed and presented as mean ± standard deviation (SD).

Detection of telomere length: Changes in telomere length were assessed as an indicator of the anti-aging effects of the samples. Following the grouping and treatment protocol described in Section 2.2, 50 zebrafish from each group were homogenized at 72 hpf. The culture solution was refreshed daily, and at 72 hpf, 30 zebrafish from each group were homogenized as described previously. DNA was extracted from each group using a DNA extraction kit. Fluorescent quantitative PCR (LongGene, Q2008, Hangzhou, China) was performed on a fluorescence PCR instrument to determine telomere lengths. The qPCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 54°C for 2 minutes. Relative telomere lengths were calculated as 1/(Cq_telomere/Cq_single_copy_gene), with the c-fos single-copy gene used as the internal reference. The

primers used for this assay are listed in Table 1.

Table 1. The sequences of the primers used in the determination of telomere lengths.

Primer	Sequence
z-q-Tel-F	GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT
z-q-Tel-R	TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA
z-q-c-fos-F	CAGCTCCACCACAGTGAAGA
z-q-c-fos-R	GCTCCAGGTCAGTGTTAGCC

Detection of mitochondrial membrane potential: Mitochondrial membrane potentials were assessed as a measure of the anti-aging effects of the samples. Following the grouping and treatment protocol described in Section 2.2, the culture medium was refreshed daily. At 72 hpf, 10 zebrafish from each group were randomly selected and incubated with the NAD+ probe kit for 45-60 minutes. The fish were then observed and photographed under a fluorescence microscope (MST, MSD530), and fluorescence intensity was quantified using ImageJ software.

Statistical Analysis: Statistical analysis was performed using GraphPad Prism 8.0.2 statistical software. All measurement data were expressed as mean ± standard deviation (Mean ± SD). Pairwise comparisons were performed using Student's t-test. All statistical analyses were conducted in a blinded manner. P-values <0.05 were considered statistically significant.

RESULTS

R-18CP reduced β-galactosidase levels in zebrafish: Significant differences in β-galactosidase staining intensity were found between the control and model groups. The model group exhibited a higher intensity, indicating a successful establishment of the aging model (Figure 1A and B). As the sample concentration increased, β-galactosidase staining intensity decreased in a dose-dependent manner, with the 1 μg/mL group showing the most significant reduction, which was significantly lower than that of the model group (Figure 1B).

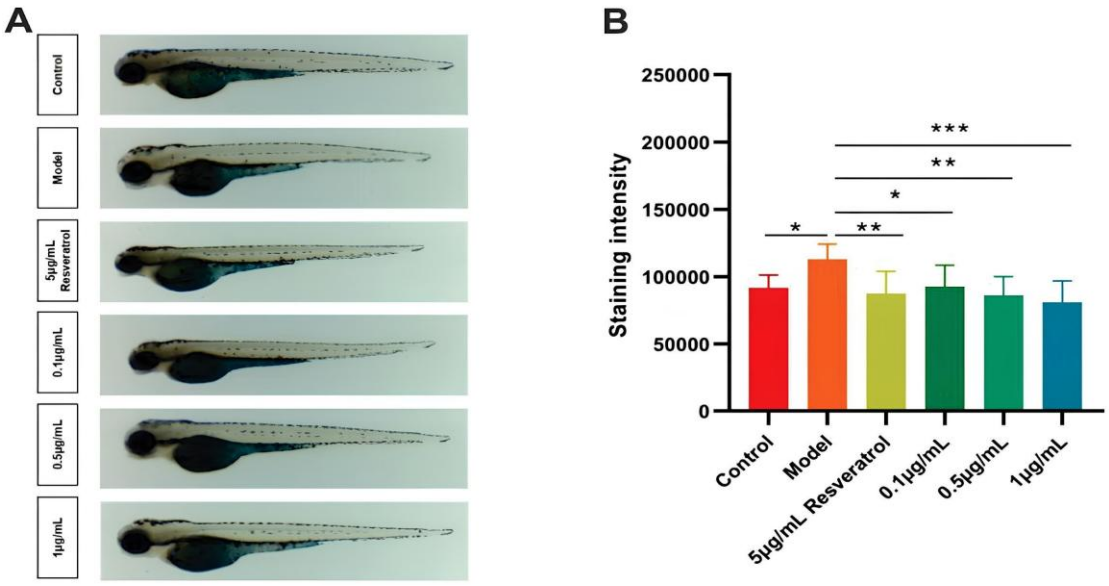


Figure 1. β-galactosidase staining following 72 hours-treatment with R-18CP in zebrafish. (A) Representative images of zebrafish from each group following staining for β-galactosidase. (B) β-galactosidase staining intensities in each group. Graph B shows mean intensity ± SD (n=10 per study group). * p<0.05, ** p<0.01, *** p<0.001 for comparison with the aging model.

R-18CP inhibited ROS production in zebrafish: We found that the fluorescence intensities of ROS in both the positive control group and the R-18CP-treated groups were significantly lower than in the model group (Figure 2A&B). Additionally, R-18CP treatment in zebrafish resulted in a dose-dependent reduction in ROS fluorescence intensity, with the decrease being significantly greater than that observed in the aging model (Figure 2B).

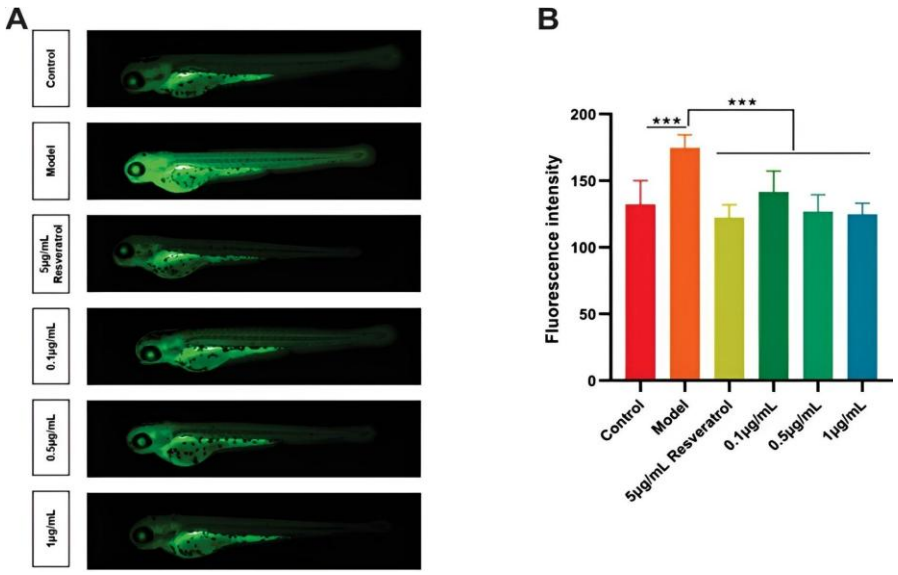


Figure 2. ROS staining following 72 hours of treatment of zebrafish with R-18CP and resveratrol. (A) Representative images of zebrafish from each group following staining for ROS. (B) Fluorescence intensities of ROS in each group. Mean ± SD (n=10 per study group). *** p<0.001 for comparison with the aging model.

R-18CP increased telomerase activity in zebrafish: We have found that the telomerase levels in the model group were significantly lower than those in the R-18CP-treated groups (Figure 3). Furthermore, treatment of zebrafish with R-18CP and resveratrol resulted in significant increases in telomerase levels in a dose-dependent manner, compared to the aging model.

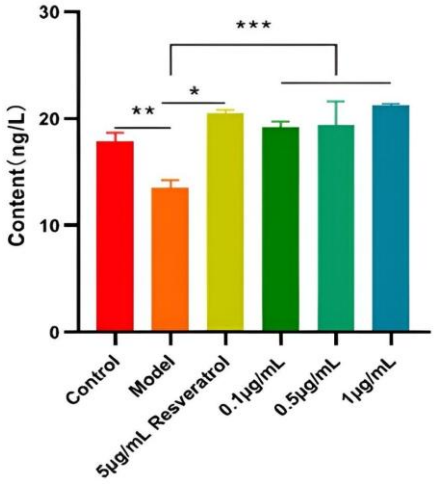


Figure 3. Telomerase activity levels (expressed as content) following 72 hours of treatment of zebrafish with R-18CP and resveratrol. The graph shows the mean ± SD (n = 10 per study group), * < 0.05, ** < 0.01, and ***p < 0.001 for comparison with the aging model.

R-18CP increased telomere length in zebrafish: The relative telomere lengths in the R-18CP-treated groups were consistently longer than those in the model group, and treatment with R-18CP resulted in a dose-dependent increase in the relative telomere length (Figure 4).

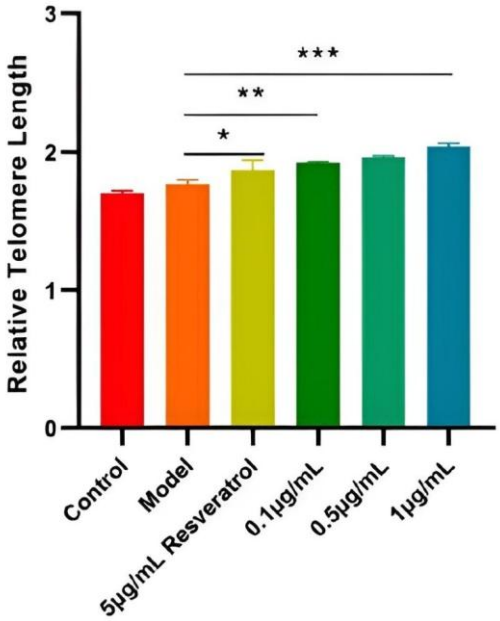


Figure 4. Relative telomere length following 72 hours of treatment of zebrafish with R-18CP and resveratrol. Relative telomere length (see Section 2.6 for the method used to quantify relative telomere length) in zebrafish for each group. The graph shows the mean ± SD (n = 10 per study group), * $p < 0.05$, ** $p < 0.01$ for comparison with the aging model.

R-18CP increased mitochondrial membrane potential in zebrafish: Compared to the aging model group, the mitochondrial membrane potential was significantly higher in both the positive control group treated with resveratrol and the groups treated with 0.5 μg/mL and 1.0 μg/mL of R-18CP (Figure 5A and B). Moreover, R-18CP treatment resulted in a dose-dependent increase in fluorescence intensity in the zebrafish (Figure 5B).

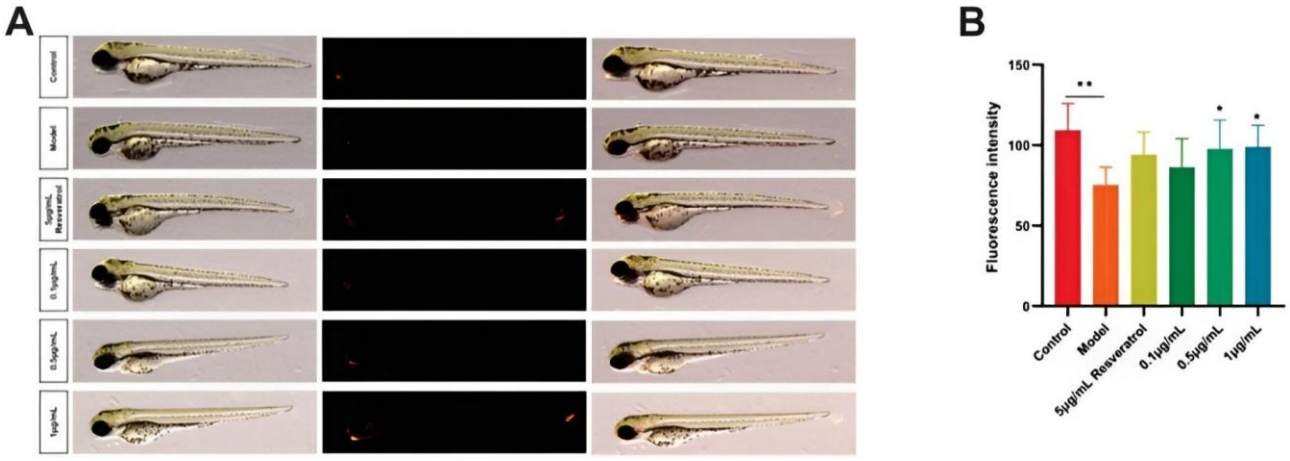


Figure 5. Mitochondrial membrane potential staining following 72 hours of treatment of zebrafish with R-18CP and resveratrol. (A) Representative images of zebrafish (left and right panels) and fluorescence images of mitochondrial membrane potential (center panels). (B) Fluorescence intensities of mitochondrial membrane potential in zebrafish in each group. Mean ± SD (n=10 per study group), * $p<0.05$, ** $p<0.01$ for comparison with the aging model.

DISCUSSION

The results from the R-18CP experiments demonstrate significant effects on aging-related cellular processes in zebrafish, offering valuable insights into its potential as an anti-aging product. The findings indicate enhanced telomerase activity and increased telomere length, suggesting a protective mechanism against oxidative stress induced by aging [15-16]. We have demonstrated that zebrafish treated with R-18CP exhibited increased telomerase activity. Interestingly, a previous study reported that treatment with the green tea polyphenol epigallocatechin gallate (EGCG) significantly shortened telomere length, accompanied by a reduction in telomerase activity in human cancer cells [17]. These opposed findings highlight the context-dependent roles of telomerase, which may be modulated by functional food components such as R-18CP. The observed mitochondrial dysfunction, characterized by a reduced membrane potential, further suggests that R-18CP may modulate mitochondrial function, which is crucial for energy production and cellular health [18]. This aligns with findings from studies on *Ganoderma lucidum*, which also highlights mitochondrial dysfunction as part of its anti-aging [19-20] and antioxidant effects [21-22]. Additionally, the telomere elongation observed in this study is consistent with the effects of green tea polyphenol EGCG on normal human fibroblast cells [17], corroborating broader research on aging mechanisms and emphasizing the critical role of telomeres in maintaining genomic stability [23-24]. Collectively, these findings suggest that R-18CP may act through multiple pathways to counteract aging processes.

The zebrafish model has become an invaluable tool for studying human diseases [6-7, 25]. With external development and accessibility at all stages, zebrafish embryos and larvae offer the advantage of real-time imaging of internal organs due to their transparency [26]. Unlike murine models, zebrafish have telomere lengths similar to those of humans, with the exact telomere maintenance mechanisms [27-28], making them a suitable candidate for studying aging processes. These features support the use of zebrafish as a practical preclinical model for the early-stage screening of anti-aging compounds and preparations, such as R-18CP, particularly in the development of functional foods and nutraceuticals. While zebrafish provide valuable insights into aging, their use as a model for human aging presents several limitations. One notable drawback is their relatively long lifespan, averaging 3 to 5 years [29], which can delay experimental outcomes compared to shorter-lived model organisms, such as C. elegans, which have a lifespan of approximately three weeks [29-30]. However, a short telomerase zebrafish model has recently been developed, which accelerates the aging process and enables the study of age-related diseases within a shorter timeframe [31].

The findings from this zebrafish study suggest that R-18CP holds significant promise as a potential therapeutic product for anti-aging applications. As a formulation derived from traditional "food-medicine" ingredients such as *Panax ginseng* and *Ganoderma lucidum*, R-18CP may serve as a natural-origin alternative for mitigating age-related physiological decline. Its ability to improve multiple aging markers, such as cellular senescence, telomerase activity, telomere length, and mitochondrial function, indicates a broad spectrum of potential benefits. While further validation through human studies is essential, these preliminary results lay the groundwork for the development of novel dietary supplements or functional foods designed to promote healthy aging. Ultimately, R-18CP could offer a proactive strategy to address age-related health challenges and support a longer-term health span. Future studies involving other anti-aging compounds, preparations, and products from traditional medicine, as well as other animal models such as mice and rats, could provide comparative evidence to understand further the mechanisms by which R-18CP regulates aging.

CONCLUSION

The present study has demonstrated that R-18CP is a promising candidate for anti-aging research, potentially exerting its effects through telomere protection and improved mitochondrial function.

Authors' Contributions: LY designed the study, analyzed data, contributed fundamental conceptualization for the research, and prepared the manuscript for this study. PFD critically revised the manuscript. Both authors read and approved the final version of the manuscript.

Competing Interests: There are no conflicts of interest to declare.

Acknowledgements and Funding: We would like to acknowledge Xiaoyu Zhu (Baihuan Biotechnology) for conducting the experiments and collecting the data. This project was supported by funding from Shenzhen Suka Health Industry Co., Ltd.

List of Abbreviations: DCFH-DA: 2',7'-dichlorofluorescin-diacetate, EGCG: epigallocatechin gallate, hpf: hours post fertilization, MTC: maximum tolerated concentration, NAD: Nicotinamide adenine dinucleotide, R-18CP: Regetting-18 complex powder , PBS: Phosphate buffered saline, PFA: p-formaldehyde, ROS: Reactive oxygen species.

REFERENCES

1. Shammas MA. Telomeres, lifestyle, cancer, and aging. Curr Opin Clin Nutr Metab Care. 2011;14(1):28–34. DOI: https://doi:10.1097/MCO.0b013e32834121b1
2. Vaiserman A, Krasnienkov D. Telomere length as a marker of biological age: State-of-the-art, open issues, and future perspectives. Front Genet. 2020; 11:630186. DOI: https://doi:10.3389/fgene.2020.630186
3. Rossiello F, Jurk D, Passos JF, d'Adda di Fagagna F. Telomere dysfunction in ageing and age-related diseases. Nat Cell Biol. 2022;24(2):135–47. DOI: https://doi.org/10.1038/s41556-022-00842-x
4. Valieva Y, Ivanova E, Fayzullin A, Kurkov A, Igrunkova A. Senescence-associated beta-galactosidase detection in pathology. Diagnostics (Basel). 2022;12(10). DOI: https://doi.org/10.3390/diagnostics12102309
5. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. Cell. 2023;186(2):243–78. DOI: https://doi.org/10.1016/j.cell.2022.11.001
6. Keller ET, Murtha JM. The use of mature zebrafish (Danio rerio) as a model for human aging and disease. Comp Biochem Physiol C Toxicol Pharmacol. 2004;138(3):335–41. DOI: https://doi.org/10.1016/B978-0-12-811353-0.00026-9.
7. White RM, Patton EE. Adult zebrafish as advanced models of human disease. Dis Model Mech. 2023;16(8). DOI: https://doi:10.1242/dmm050351.
8. Cayuela ML, Claes KBM, Ferreira MG, Henriques CM, van Eeden F, Varga M, et al. The zebrafish as an emerging model to study DNA damage in aging, cancer and other diseases. Front Cell Dev Biol. 2018; 6:178. DOI: https://doi:10.3389/fcell.2018.00178
9. Khor ES, Noor SM, Wong PF. Understanding the role of ztor in aging-related diseases using the zebrafish model. In Vivo. 2019;33(6):1713–20. DOI: https://doi.org/10.21873/invivo.11661
10. Wang L, Zhu X, Liu H, Sun B. Medicine and food homology substances: A review of bioactive ingredients, pharmacological effects and applications. Food Chem. 2025;463(Pt 1):141111. DOI: https://doi.org/10.1016/j.foodchem.2024.141111
11. Kang M, Park S, Son SR, Noh Y, Jang DS, Lee S. Anti-aging and anti-inflammatory effects of compounds from fresh panax ginseng Roots: A study on TNF-alpha/IFN-gamma-induced skin cell damage. Molecules. 2024;29(22). DOI: https://doi.org/10.3390/molecules29225479
12. Pan M, Wu Y, Sun C, Ma H, Ye X, Li X. Polygonati Rhizoma: A review on the extraction, purification, structural characterization, biosynthesis of the main secondary metabolites and anti-aging effects. J Ethnopharmacol. 2024; 327:118002. DOI: https://doi.org/10.1016/j.jep.2024.118002
13. Zhou DD, Luo M, Huang SY, Saimaiti A, Shang A, Gan RY, et al. Effects and mechanisms of resveratrol on aging and age-related diseases. Oxid Med Cell Longev. 2021:9932218. DOI: https://doi.org/10.1155/2021/9932218
14. Luo, L, Yan, T, Le Yang, L, Zhao, M. Aluminum chloride and D-galactose induced a zebrafish model of Alzheimer's disease with cognitive deficits and aging. Comput. Struct Biotech J 23 2024: 2230–9. DOI: https://doi.org/10.1016/j.csbj.2024.05.036

15. Chakravarti D, LaBella KA, DePinho RA. Telomeres: history, health, and hallmarks of aging. Cell. 2021;184(2):306–22. DOI: https://doi.org/10.1016/j.cell.2020.12.028
16. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194–217. DOI: https://doi.org/0.1016/j.cell.2013.05.039
17. Pointner, A, Mölzer, C, Magnet, U, Zappe,K, Hippe, B, E, Tosevska, A, et al. The green tea polyphenol EGCG is differentially associated with telomeric regulation in normal human fibroblasts versus cancer cells. Functional Foods in Health and Disease. 2021;11(3):73–91. DOI: https://doi.org/10.31989/ffhd.v11i3.775
18. Kudryavtseva AV, Krasnov GS, Dmitriev AA, Alekseev BY, Kardymon OL, Sadritdinova AF, et al. Mitochondrial dysfunction and oxidative stress in aging and cancer. Oncotarget. 2016;7(29):44879–905. DOI: https://doi.org/10.3390/ijms242015483
19. Ajith TA, Sudheesh NP, Roshny D, Abishek G, Janardhanan KK. Effect of Ganoderma lucidum on the activities of mitochondrial dehydrogenases and complex I and II of electron transport chain in the brain of aged rats. Exp Gerontol. 2009;44(3):219–23. DOI: https://doi.org/10.1016/j.exger.2008.11.002
20. Wang J, Cao B, Zhao H, Feng J. Emerging roles of ganoderma lucidum in anti-aging. Aging Dis. 2017;8(6):691–707. DOI: https://doi.org/10.14336/AD.2017.0410
21. Smina TP, Mathew J, Janardhanan KK, Devasagayam TP. Antioxidant activity and toxicity profile of total triterpenes isolated from Ganoderma lucidum (Fr.) P. Karst occurring in South India. Environ Toxicol Pharmacol. 2011;32(3):438–46. DOI: https://doi.org/10.1016/j.etap.2011.08.011
22. Kao, C, Jesuthasan, AC, Bishop, KS, Glucina, MP, Ferguson, LR. Anti-cancer activities of *Ganoderma lucidum*: active ingredients and pathways. Functional Foods in Health and Disease. 2013;3(2):48–65. DOI: https://doi.org/10.31989/ffhd.v3i2.65
23. Zhou B, Kreuzer J, Kumsta C, Wu L, Kamer KJ, Cedillo L, et al. Mitochondrial permeability uncouples elevated autophagy and lifespan extension. Cell. 2019;177(2):299–314 e16. DOI: https://doi.org/10.1016/j.cell.2019.02.013
24. Penberthy WT, Shafizadeh E, Lin S. The zebrafish as a model for human disease. Front Biosci. 2002;7: d1439–53. DOI: https://doi.org/10.2741/penber
25. Ou, Y, Zhang, W, Lan, J, Huang, X Li, N. A combination of multi-strain probiotics, prebiotic, and plant extracts improves ethanol-induced hangover outcomes in a zebrafish model. Functional Foods in Health and Disease. 2024;14(10):728–38. DOI: https://doi.org/10.31989/ffhd.v14i10.1462
26. Patton EE, Zon LI, Langenau DM. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. Nat Rev Drug Discov. 2021;20(8):611–28. DOI: https://doi.org/
27. Anchelin M, Murcia L, Alcaraz-Perez F, Garcia-Navarro EM, Cayuela ML. Behaviour of telomere and telomerase during aging and regeneration in zebrafish. PLoS One. 2011;6(2): e16955. DOI: https://doi.org/10.1038/s41573-021-00210-8
28. Henriques CM, Ferreira MG. Consequences of telomere shortening during lifespan. Curr Opin Cell Biol. 2012;24(6):804–8. DOI: https://doi.org/10.1016/j.ceb.2012.09.007
29. Kishi S, Slack BE, Uchiyama J, Zhdanova IV. Zebrafish as a genetic model in biological and behavioral gerontology: where development meets aging in vertebrates--a mini-review. Gerontology. 2009;55(4):430–41. DOI: https://doi.org/10.1159/000228892
30. Zhang S, Li F, Zhou T, Wang G, Li Z. Caenorhabditis elegans as a Useful Model for Studying Aging Mutations. Front Endocrinol (Lausanne). 2020; 11:554994. DOI: https://doi.org/10.3389/fendo.2020.554994
31. Hernandez-Silva D, Lopez-Abellan MD, Martinez-Navarro FJ, Garcia-Castillo J, Cayuela ML, Alcaraz-Perez F. Development of a short telomere zebrafish model for accelerated aging research and antiaging drug screening. Aging Cell. 2025;24(6):e70007. DOI: https://doi.org/10.1111/acel.70007