Science World Journal Vol. 19(No 3) 2024 https://dx.doi.org/10.4314/swj.v19i3.5
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

THE PHENOLIC-RICH FRACTION OF *TERMINALIA CATAPPA* MODULATED ANTIOXIDANT INDICATORS AND ENHANCED *DROSOPHILA MELANOGASTER*'S LIFESPAN

Mohammed Sani Jaafaru*, Zainab Kasim Mohammed, Richard Auta

Department of Biochemistry, Faculty of Life Science, Kaduna State University, 2339, Tafawa Balewa way, Kaduna

*Corresponding Author Email Address: biojafar@kasu.edu.ng or biojafar@gmail.com

ABSTRACT
The presence of phenolics and flavonoids in fruits and vegetables is well recognized for their potent preventative effects against age-related disorders, mostly owing to their abundant hydroxyl groups. This study examined the potential enhancement of neuromuscular function and anti-aging effects of a diet supplemented with a fraction rich in polyphenols from *Terminalia catappa* in *Drosophila melanogaster* model. Three-day-old flies of both sexes were supplemented with a diet rich in polyphenols for seven days. Upon establishment of the effective doses, an experiment was performed to assess the impact of the fraction on the lifespan, antioxidant capacity, and the process of aging of the flies. The procedures consist of the lifespan determination assay, the behavioral assay, and the biochemical assay. The study found that *D. melanogaster* flies that were fed a diet with a phenolics-rich fraction at concentrations of 2.0 mg/g and 4.0 mg/g lived longer and emerged more often than the control group. Additionally, these flies exhibited significantly reduced activity of acetylcholinesterase (AChE). The activity of catalase, superoxide dismutase (SOD), and glutathione-s-transferase (GST) in the experimental flies was also elevated by the fraction-supplemented diet in a concentration-dependent manner. The phenolics derived from *T. catappa*, exhibited robust biological activities and caused the experiment's discernible changes. The fraction strengthened the flies' antioxidant system by increasing the activities of several phase II antioxidant enzymes in *D. melanogaster*. The present research provides a better understanding of the wider society's viewpoints on the possible use of plant-derived natural chemicals to avert aging and age-related ailments, thereby enhancing the well-being and standard of living for both animals and humans.

Keywords: Antiaging, Antioxidants, *Drosophila melanogaster*, Flavonoids, Phenolics, *Terminalia catappa*.

1. INTRODUCTION

Aging, a multifaceted phenomenon, commonly manifests as a gradual accumulation of molecular alterations over time, leading to increased vulnerability to illness and mortality (Li *et al*., 2023). The increasing age of the global population has led to a higher occurrence of neurodegenerative diseases, including Alzheimer's and Parkinson's, which pose a substantial health concern (Bouvier *et al*., 2022; Bazzani *et al*., 2022). Mitochondria play a vital role in generating reactive oxygen species (ROS) and nitrogen species (RNS), which can alter the body's redox status (Bazzani *et al*., 2022). The progressive oxidation of macromolecules results in the generation of reactive oxygen species (ROS) at levels that exceed pathogenic thresholds (Jaafaru et al., 2018). Oxidative stress results from this situation results in mitochondrial damage and ultimately causes cell death through apoptosis (Bazzani *et al*., 2022; Zheng *et al*., 2023). Studies have shown that the production of reactive oxygen species (ROS) and the resulting response to oxidative stress are important factors that influence lifespan (Vagasi *et al*., 2019; Abdulwanis *et al*., 2020; Amorim *et al*., 2022). Environmental and genetic factors impact a multitude of molecular pathways and biochemical events that govern the aging process (Sharma and Diwan, 22023). The process of aging is characterized by a progressive decline in functional capacity and resistance to stress. This decline is accompanied by an increased risk of developing illnesses and ultimately death (Zhang *et al*., 2023). The consequences are linked to the gradual buildup of stressors that are characteristic of the aging process, which results in the gradual degradation of biomolecules and consequent disturbance of cellular homeostasis (Brandl *et al*., 2023). Nevertheless, prior research has indicated that genetic or dietary modifications possess the capacity to prolong the existence of various organisms (Yuan *et al*., 2020; Molon *et al*., 2020; Jin *et al*., 2022). This suggests that mortality can be delayed via such interventional strategies.

Lifespan extension and slowing down the aging process are key healthcare concerns in recent years. The natural compounds, a collection of structurally diverse phytochemicals are composed of considerable potential entities that could address this issue (Herath *et al*., 2021; GomezGarcia and Medina-Franco, 2022). The medicinal plant *Terminalia catappa*, also known as Indian almond, is rich in bioactive compounds. In sub-Saharan Africa, *T. catappa* is highly valued for its culinary and medicinal uses (Abdelnaby *et al*., 2022; Saxena *et al*., 2022). The fruit of the plant is rich in bioactive phytochemicals, including bioflavonoids, alkaloids, and phenolic acids (Sarkar *et al.,* 2020; Saxena *et al*., 2022). Polyphenolics, the main phytomolecules found in the plant's fruit, particularly ripe fruit, have been the major focus of this research. Its pharmacological effects have been studied in many model organisms, and some researchers have even suggested that it could be considered for clinical trials (Xie *et al*., 2023; Chen *et al*., 2023).

Drosophila melanogaster is a widely used model organism in biomedical research, specifically in the investigation of genetic pathways involved in human diseases (Mishra and Thakur, 2023; Mishra *et al*., 2023; Baenas and Wagner, 2022). The model has been widely utilized for biochemical research endeavours. Furthermore, the fly model has shown a notable resemblance in neurotoxicity to Homo sapiens (humans) (Mohammed *et al*., 2022; Rouka *et al*., 2022). Scientists utilized the simple brain network of the flies to study the antioxidant capabilities of bioactive

compounds that could potentially provide neuroprotective benefits (Deolankar *et al*., 2023). The fly model is highly effective in unraveling the mysteries of life at a molecular level and assessing the efficacy of possible therapeutic drugs (Luna *et al*., 2021). The goal of the present study was to assess the anti-aging ability of a phenolic-rich fraction from *T. catappa* ripped fruit and its capacity to prolong *D. melanogaster*'s lifespan.

2.0 MATERIALS AND METHODS

2.1 Sample collection and preparation.

A sample of mature fruit from *T. catappa* was obtained from the Kaduna metropolis in Nigeria, which was identified with the Boucher number K6615 by a botanist. A stainless-steel blender was used to pulverize the pulp sections of the fruit into finely powdered particles after drying. The powdered sample was immersed in ethanol for 72 hours and filtered using Wattman paper. The filtrate was evaporated using a rotary evaporator until it was completely dried up. The resulting product had a yield of 8.2% w/w and was stored in the refrigerator until it was required.

2.2 Diet formulation and culture of the flies

The Harwich strain of *Drosophila melanogaster* was graciously provided by the College of Medicine, University of Ibadan, Nigeria. The flies model, which was initially obtained from the National Species Stock Centre in Bowling Green, Ohio, USA, was cultivated in the *Drosophila* Research Laboratory, Kaduna State University. The flies were kept at a consistent temperature of 24 ± 2 °C and a relative humidity of 60 – 70% over a 12-hour cycle of light and darkness. Their food included cornmeal with the following concentrations: 0.08% w/v methylparaben, 1% w/v agar–agar, 1% w/v brewer's yeast, and 2% w/v sucrose.

2.3 Experimental Plan

Male and female flies, aged two to three days, were categorized into three separate groups: a control group (fed a diet without the fraction), treatment group 1 (fed a diet containing 2.0 mg of the phenol-rich fraction per gram of diet), and treatment group 2 (fed a diet containing 4.0 mg of the phenol-rich fraction per gram of diet). The two doses were identified as the most efficacious concentrations of the fractions. The experiment was conducted in triplicates with each vial comprised of 100 flies, which were fed for seven days.

2.4 Behavioural Assays

2.4.1 Longevity Assay

To determine the effect of the phenolic-rich fraction on the longevity of experimental *D. melanogaster*, 100 flies contained in a vial were treated in triplicate for seven days at concentrations of 2.0 and 4.0 mg/g, with or without the fraction. The flies' daily mortality was monitored and documented throughout seventy-seven (77) days, and the GraphPad Prism analysis of the survival rate was detailed in the results section.

2.4.2 Climbing Assay

The negative geotaxis assay, as described by (Nichols *et al*., 2012). with some modifications, was employed to evaluate the locomotor activity of *D. melanogaster* that was fed the fraction-supplemented diet. Twenty flies from each vial were selected and subjected to a graduated column measuring 15 cm in height and 1.5 cm in diameter following ice-based anesthesia. The flies' strength was measured by climbing a threshold of 8 cm height. The number of flies that crossed the threshold and the number of flies that remained at the bottom within 8 seconds were both recorded. The data were analyzed and presented in the result section. Meanwhile, the whole process was repeated three times per vial with a one-minute interval between readings.

2.4.3 Determination of flies' progeny rate of emergence.

The emergence rate of flies' progeny in the treatment group supplemented with a phenolic-rich fraction was assessed following the methodology specified in (Arias, 2008).

2.5 Bioassay of aging and antioxidant markers in *Drosophila melanogaster*

2.5.1 Quantification of acetylcholinesterase enzymatic activity

The measurement of AChE activity was carried out using the procedure outlined by (Worek *et al*., 1999) with some modifications. Summarily, a reaction mixture consisting of 135 µL of deionized water, 20 µL of 100 mM potassium phosphate buffer (pH 7.4), 20 µL of 10 mM DTNB, 5 µL of homogenate sample, and 20 µL of 8 mM ACh substrate was vigorously shaken. The enzymatic function of acetylcholinesterase was monitored using a UV/visible spectrophotometer for 5 minutes, with measurements taken at 15-second intervals, specifically at a wavelength of 412 nm. The obtained results were adjusted utilizing protein content by computation with blank and sample blank.

2.5.2 Quantification of catalase activity

The catalase activity was measured using a modified method described by (Vives-Bauza *et al.,* 2007). The reaction vessel consists of 1800 µL of a 50 mM phosphate buffer (pH 7.0), 20 µL of a homogenate sample diluted at a ratio of 1:50, and 180 µL of a 300 mM hydrogen peroxide (H_2O_2) substrate. The substrate's disappearance was observed over 2 minutes, with measurements taken every 10 seconds using a UV/visible spectrophotometer set to a wavelength of 240 nm. The results were quantified as the micromoles of H_2O_2 used per minute per milligram of protein.

2.5.3 Quantification of superoxide dismutase (SOD) activity

The superoxide dismutase (SOD) activity was assessed using the procedures described in (Vives-Bauza *et al*., 2007), with minor adjustments. This involved measuring the reduction in nitrite production over 40 minutes at a temperature of 37 °C. The test depended on SOD's suppression of the production of nitrite from hydroxyl ammonium when O_2 generators were present. The activity was quantified using spectrophotometry at a wavelength of 550 nm. The findings were then expressed as the enzyme's activity per milligram of protein.

2.5.4 Quantification of glutathione-s-transferase activity

The activity of glutathione-s-transferase was evaluated by closely monitoring the rise in absorbance at a wavelength of 340 nm according to the method described by (Prohaska, 1980) 50 µL of the material was introduced into a tube that already contained 20 µM of both 1-chloro-2,4-dinitrobenzene (CDNB) and a reduced version of glutathione. The optical density was measured at a wavelength of 406 nm for three minutes. The outcome was reported as the amount of protein required to prevent fifty percent of the quercetin auto-oxidation.

Science World Journal Vol. 19(No 3) 2024 https://dx.doi.org/10.4314/swj.v19i3.5
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

2.6 Statistical Analysis

Mean ± SD was used in reporting the present findings, following the conduction of statistical analysis on version 9.5.1 of GraphPad Prism using analysis of variance (ANOVA) and Tukey post hoc test. The observed discrepancies in the outcomes were deemed statistically significant ($p < 0.05$) with a 95% level of confidence. The experiments were carried out in triplicates (n = 3).

3. RESULTS

3.1 Phenolic-rich fraction modulates lifespan of *D. Melanogaster*

In comparison to the control group, which only received a regular diet (without a fraction), administering the phenolic-rich fraction of *T. catappa* over thirty days resulted in a considerable extension of *D. melanogaster*'s lifespan. The impact was dependent on the concentration, as the flies that were fed a 4.0 mg/g fraction rich in phenolics showed a higher lifespan extension compared to those that received 2.0 mg of the fraction. Nevertheless, the disparity between the two doses was not significant, as seen in Figure 1.

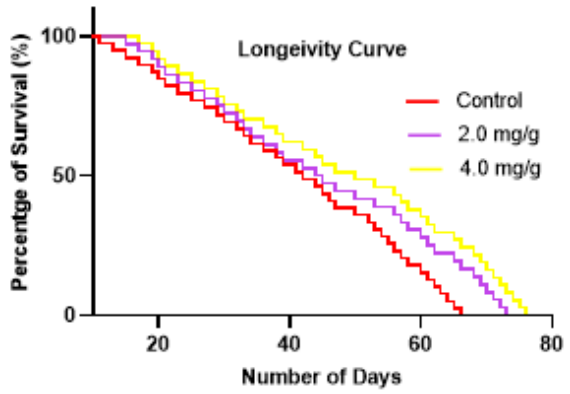

Figure 1: shows the impact of a diet-supplemented phenolics-rich fraction of *T. catappa* on the lifespan of *D. Melanogaster*. The studies were done in three biological and experimental replicates (n=3).

3.2 Phenolics-rich fraction influences locomotor function and emergence rate of *D. melanogaster.*

Figure 2 illustrates the impact of a diet supplemented with phenolic-rich fractions on the flies' emergence rate and locomotor activity. When added to the diet at both concentrations (2.0 and 4.0 mg fraction/g diet), the phenolic-rich fraction greatly increased the number of new flies compared to the control groups ($p < 0.05$). The observed results showed a dose-dependent pattern, with a concentration of 4.0 mg/g having a greater effect than 2.0 mg/g, as shown in Figure 2a. Additionally, the flies fed with the phenolic fraction at 4.0 mg/g of diet showed a considerable increase in their locomotor activity (Figure 2b). The flies' locomotor activity also experienced a dose-dependent increase, as evidenced by the comparison.

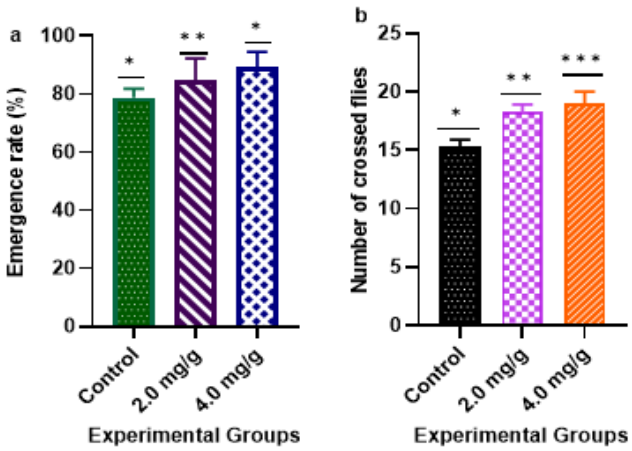

Figure 2: Illustration of the impact of a diet-supplemented fraction rich in phenolics from *T. catappa* on the rate of flies' emergence (a) and locomotor function (b) of *D. melanogaster*. The result for the emergence rate was presented as the percentage pupation of new flies fed with and without the fraction. The locomotor function was presented as the mean ± SD of the number of flies that crossed a threshold mark during the experiment. The studies were done in three biological and experimental replicates (n = 3). The observed differences in the outcomes were deemed to be statistically significant ($p < 0.05$) with a confidence level of 95%.

3.3 Phenolics-rich fraction regulates age-related enzyme activities in *D. melanogaster.*

The fraction's inhibitory effect on the activity of acetylcholinesterase in *D. melanogaster* was further assessed in relation to aging-related enzymes. Compared to the normal control, only 4.0 mg of fraction per gram of diet demonstrated a significant difference ($p < 0.05$). (Figure. 3).

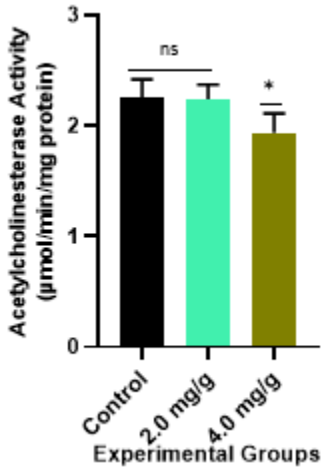

Figure 3: Illustration of the impact of a diet supplemented with a fraction rich in phenolics from *T. catappa* on the activity of *D. melanogaster's* acetylcholinesterase. The finding is reported as the mean value plus or minus the standard deviation (SD) for the enzyme's activity. The studies were done in three biological and experimental replicates (n = 3). The observed disparities in the outcomes were deemed to be statistically significant ($p < 0.05$) at a 95% level of confidence.

Science World Journal Vol. 19(No 3) 2024 https://dx.doi.org/10.4314/swj.v19i3.5
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

3.4 Phenolics-rich fraction regulates antioxidant enzymes' activities in *D. melanogaster.*

Figure 4 illustrates the impact of a diet supplemented with a fraction rich in phenolics from *T. catappa* ripped fruit on certain antioxidant indicators in *D. melanogaster*. The fraction at a concentration of 4.0 mg/g caused a substantial ($p < 0.05$) increase in catalase activity in the flies compared to the normal control group that was fed without the fraction. Nevertheless, the activity did not show any notable impact when the flies were fed with a 2.0 mg fraction/g of diet (Fig. 4a). The findings also indicated a statistically significant ($p < 0.05$) rise in superoxide dismutase (SOD) activity in the flies fed with the fraction-supplemented diet, as compared to the flies in the normal control group. The two concentrations of the added fraction of *T. catappa* had similar effects on the SOD activity, as their averages were not significantly different (Fig. 4b). In the same vein, a significant rise ($p<0.05$) in the GST activity in the flies due to the presence of the fraction was noticed. Comparing the two concentrations with the normal control revealed a dose-dependent effect. The effect of 2.0 mg/g of diet on GST activity was significantly lower as compared with 4.0 mg/g of diet (Figure 4c).

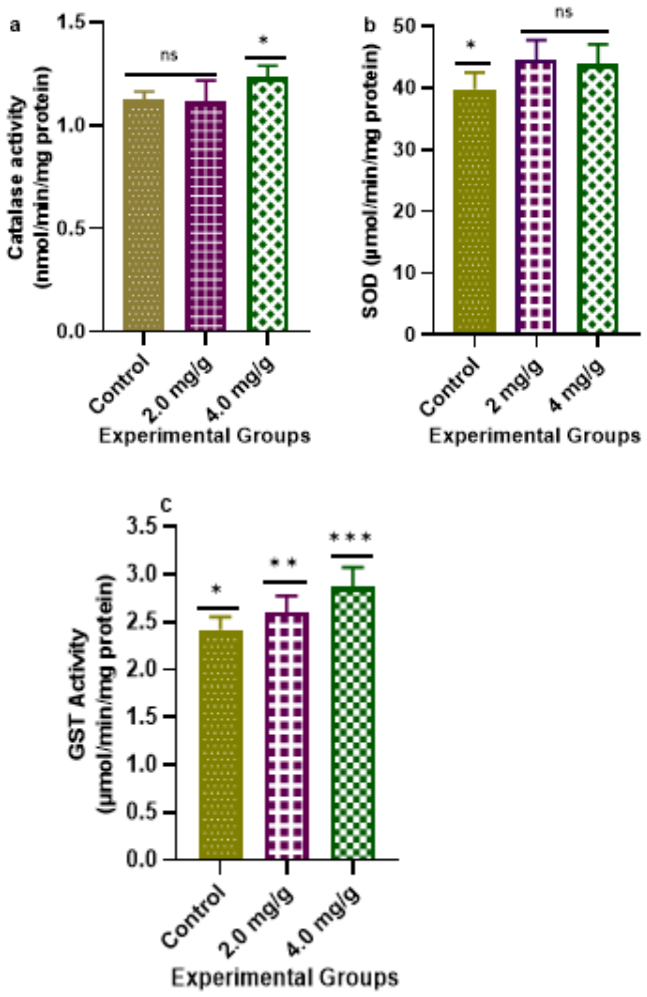

Figure 4: Illustration of the impact of a diet supplemented with a fraction rich in phenolics from *T. catappa* on the antioxidant enzymes' activity of *D. melanogaster's* catalase (a), superoxide dismutase (b) and glutathione-s-transferase (c). The findings were reported as the mean value plus or minus the standard deviation (SD). The studies were done in three biological and experimental replicates (n = 3). The observed disparities in the outcomes were deemed to be statistically significant ($p < 0.05$) at a 95% level of confidence.

DISCUSSION

Researchers have studied the impact of different flavonoids and polyphenols on increasing longevity, improving health, and reducing age-related diseases using the *Drosophila melanogaster* model (Gua *et al*., 2019; Panchenko *et al*., 2019; Wang *et al*., 2020). Nevertheless, the precise influence of phenolics on lifespan, oxidative balance, and age-related diseases has yet to be fully understood. Phenolics have shown many health advantages in different model species (Chen *et al*., 2023; Zhang *et al*., 2023). Oxidative stress has been identified as a contributing factor to the aging process and the onset of many age-related illnesses (Kristiani and Kim, 2023). Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the capacity of the cellular antioxidant defense system to neutralize these substances (Korovesis *et al*., 2023). This situation occurs when reactive oxygen species or reactive nitrogen species levels increase or decrease antioxidant capability (Korovesis *et al*., 2023). Concurrently, the harmful effects on lipids, proteins, and DNA in many tissues impact the process of aging (Adeyemi *et al*., 2016). The inclusion of phenolic fraction in the diet has been demonstrated to increase the lifespan of flies in comparison to those that do not receive the fraction, as indicated by the results of this study (Figure. 1). The present results are consistent with previous research that has shown the life-extending properties of phenolics, and other forms of flavonoids found in fruits (Wang *et al*., 2023; Warnsmann *et al*., 2018; Yang *et al*., 2021). Vegetables and various forms of other fruits, including *T. catappa,* predominantly contain strong bioactive compounds known as phenolics and flavonoids (Fagbemi *et al*., 2022). The hydroxyl groups in their aromatic ring structures and the presence of highly activated carbon atoms between the two methoxyphenol rings are the reasons for the antioxidant properties that have been demonstrated (Wang *et al*., 2020; Fagbemi *et al*., 2022). Conversely, many studies have shown that the cholinergic system are important part of how neurodegenerative diseases and aging affect the body (Abate *et al.,* 2020; Bamshad *et al.,* 2023). Cholinergic cells produce and secrete acetylcholine (ACh), a biochemical molecule essential for regulating cholinergic functions such as memory, learning, and locomotion (Graur *et al*., 2023; Nascimento *et al*., 2022). However, acetylcholinesterase (AChE), a serine protease enzyme, breaks down acetylcholine into choline and acetate, which changes the way cholinergic neurons communicate in the brain and spinal cord (Nascimento *et al*., 2022). The cholinergic marker enzyme (AChE) is crucial for maintaining acetylcholine levels in the cholinergic neurons. When the level exceeds the threshold, the enzyme is also responsible for acetylcholine degradation in the synaptic cleft. It is highly specific to the active state of cholinergic neurons (Taylor *et al*., 2021). The aging process and other neurodegenerative diseases have been associated with AChE activities (Lista *et al*., 2023).

In comparison to the control group, the supplemented diet rich in phenolic fractions led to a substantial reduction of AChE activity in

D. melanogaster (Figure 3a). The effect also modulated the flies' climbing activity (Figure 2b), resulting in an improvement in neuromuscular strength in the experimental flies. These results are in accordance with prior reports on *in vitro* and *in vivo* findings (Tallini *et al*., 2023; Durmaz *et al*., 2022; Amirahmadi *et al*., 2021; Cardoso *et al*., 2020; Ferreira *et al*., 2020). Therefore, the decrease in AChE activity after dietary phenolics supplementation in our study could lead to a rise in acetylcholine levels in the synaptic cleft, which would then make cholinergic neurotransmission more effective in the flies. The present study illustrates that the antioxidant status of *D. melanogaster* is also improved by the incorporation of phenolic-rich fractions in the diet, as seen in Figure 4. The antioxidative properties of phenolics, flavonoids, and other significant phytochemicals have been demonstrated in numerous studies (Muflihah *et al*., 2021; Pauliuc *et al*., 2020; Jaafaru *et al*., 2018; Qiu *et al*., 2023). One way that living things protect themselves from the harmful effects of reactive oxygen and nitrogen species (ROS and RNS) is by increasing the activity of different markers, including catalase, superoxide dismutase (SOD), and glutathione-s-transferase enzymes (Saxena *et al*., 2022; Yang *et al*., 2021; Forman and Zhang, 2021). Superoxide dismutase (SOD) facilitates the conversion of superoxide ions into less hazardous compounds, which catalase catalytic activity subsequently converts to water (He *et al.*, 2021). Numerous reports have detailed the importance of this mechanism in the lifespan of *D. melanogaster* (He *et al.*, 2021; Liu, 2022; Jaafaru *et al*., 2024). The genome of *D. melanogaster* contains four regions that can suppress the activity of SOD and catalase, as well as individual, isolated regions that exhibit the ability to enhance their respective activities (Deepashree *et al*., 2022; Abolaji *et al*., 2020). Currently, feeding *D. melanogaster* the phenolic-rich fraction significantly alters the activities of SOD and catalase compared to the control group (Figures 4a and 4b). Our findings align with the findings of (Semaniuk *et al*., 2022), which showed an increase in SOD and catalase activities in fruit flies fed polyphenolic (curcumin) compared to those fed the control diet. Glutathione-s-transferase (GST) is an additional significant antioxidant marker. Cysteine-rich domains distinguish it from the other members of the phase II group of multifunctional enzymes (Potega, 2022). The ability of GST to speed up the conjugation of glutathione (GSH) with electrophilic molecules is a key step in getting rid of xenobiotics, which are chemicals that can harm living things' redox balance (Potega, 2022). The present results illustrated the beneficial impact of the phenolic-rich fraction on GST activities in flies that were fed the fraction in comparison to the control group. Curcumin substantially enhanced the activity of GST, even in a noxious environment. It also counteracts the detrimental impact of the ecotoxic agent, which modifies the redox status of an organism (Semaniuk *et al*., 2022).

Conclusion

The collective findings suggest that the aging process in flies is slowed by the fraction rich in phenolic compounds. As evidenced by the observed increase in the activities of certain phase II antioxidant enzymes, the anti-aging and lifespan extension ability of phenolic-rich fraction is hypothesized to be due to its antioxidative properties. This is supported by the subsequent reduction in acetylcholinesterase's (AChE) activity and modulation of the internal antioxidant marker enzymes' activities in *D. melanogaster*. Consequently, the phenolics-rich fraction from *T. catappa* could be regarded as a promising anti-aging intervention and may offer protection for the nervous system and neuromuscular disorders implicated in oxidative stress, including but not limited to Parkinson's and Alzheimer's diseases. Additionally, the results of the present study offer further evidence that *Drosophila melanogaster* is a valuable model organism for the investigation of potential newly discovered therapeutics that could improve the quality of life in the aged population.

Abbreviations

Acetylcholinesterase (AChE)
Acetylcholine (ACh)
Analysis of variance (ANOVA)
Glutathione-s-transferase (GST)
Superoxide dismutase (SOD)
Reactive oxygen species (ROS)
Reactive nitrogen species (RNS)
Phenylmethanesulfonylfluoride (PMSF)
Dithiothreitol (DTT)
Ultraviolet (UV)
Hydrogen peroxide (H_2O_2)
Oxygen (O_2)
Dichlorophenolindophenol (DCPIP)

Acknowledgments

The author expressed their gratitude to the Tertiary Education Trust Fund (TETFund) Nigeria for sponsoring the research through the Institutional Base Research (IBR) Grant for Kaduna State University with reference number **TETF/DR&D/CE/UNIV/KADUNA/IBR/2018/VOL.I**

Authors Contributions

JMS formulated the idea and planned the experiment; JMS and ZKM conducted the experiment and examined the results; JMS and RA composed and reviewed the paper. Each of the writers has reviewed and given their approval to the final version of the document.

Funding

The current research was financially supported by Nigeria's Tertiary Education Trust Fund (TETFund) via the Institutional Base Research (IBR) Fund.

REFERENCES

Abate, G., Vezzoli, M., Sandri, M., Rungratanawanich, W., Memo, M. and Uberti, D., 2020. Mitochondria and cellular redox state on the route from ageing to Alzheimer's disease. *Mechanisms of Ageing and Development*, *192*, p.111385. https://doi.org/ 10.1016/j.mad.2020.111385

Abdelnaby, A., Abdel-Aleem, N., Mansour, A., Abdelkader, A., Ibrahim, A.N., Sorour, S.M., Elgendy, E., Bayoumi, H., Abdelrahman, S.M., Ibrahim, S.F. and Alsaati, I., 2022. The combination of Tamarindus indica and coenzyme Q10 can be a potential therapy preference to attenuate cadmium-induced hepatorenal injury. *Frontiers in Pharmacology*, *13*, p.954030. https://doi.org/ 10.3389/fphar.2022.954030

Abdulwanis Mohamed, Z., Mohamed Eliaser, E., Jaafaru, M.S., Nordin, N., Ioannides, C. and Abdull Razis, A.F., 2020. Neuroprotective Effects of 7-Geranyloxycinnamic Acid from Melicope lunu ankenda Leaves. *Molecules*, *25*(16), p.3724. https://doi.org/10.3390/molecules25163724

Abolaji, A.O., Fasae, K.D., Iwezor, C.E., Aschner, M. and Farombi,

E.O., 2020. Curcumin attenuates copper-induced oxidative stress and neurotoxicity in Drosophila melanogaster. *Toxicology reports*, *7*, pp.261-268. https://doi.org/10. 1016/j.toxrep.2020.01.015

Adeyemi, K.D., Shittu, R.M., Sabow, A.B., Ebrahimi, M. and Sazili, A.Q., 2016. Influence of diet and postmortem ageing on oxidative stability of lipids, myoglobin and myofibrillar proteins and quality attributes of gluteus medius muscle in goats. *PloS one*, *11*(5), p.e0154603. https://doi.org/10. 1371/journal.pone.0154603

Amirahmadi, S., Hosseini, M., Ahmadabady, S., Akbarian, M., Abrari, K., Vafaee, F. and Rajabian, A., 2021. Folic acid attenuated learning and memory impairment via inhibition of oxidative damage and acetylcholinesterase activity in hypothyroid rats. *Metabolic Brain Disease*, *36*, pp.2393-2403. https://doi. org/10.1007/s11011-021-00815-3

Amorim, J.A., Coppotelli, G., Rolo, A.P., Palmeira, C.M., Ross, J.M. and Sinclair, D.A., 2022. Mitochondrial and metabolic dysfunction in ageing and age-related iseases. *Nature Reviews Endocrinology*, *18*(4), pp.243-258. https://doi.org/10.1038/ s41574-021-00626-7.

Arias, A.M., 2008. Drosophila melanogaster and the development of biology in the 20th century. *Drosophila: Methods and Protocols*, pp.1-25. https://doi.org/ 10.1007/978-1-59745-583-1_1

Baenas, N. and Wagner, A.E., 2022. Drosophila melanogaster as a model organism for obesity and type-2 diabetes mellitus by applying high-sugar and high-fat diets. *Biomolecules*, *12*(2), p.307. https://doi.org/10.3390/biom12020307

Bamshad, C., Najafi-Ghalehlou, N., Pourmohammadi-Bejarpasi, Z., Tomita, K., Kuwahara, Y., Sato, T., Feizkhah, A., Roushnadeh, A.M. and Roudkenar, M.H., 2023. Mitochondria: how eminent in ageing and neurodegenerative disorders?. *Human Cell*, *36*(1), pp.41-61. https://doi.org/10.1007/s13577-022-00833-y

Bazzani, V., Equisoain Redin, M., McHale, J., Perrone, L. and Vascotto, C., 2022. Mitochondrial DNA repair in neurodegenerative diseases and ageing. *International Journal of Molecular Sciences*, *23*(19), p.11391. https://doi.org/10.3390/ijms231911391

Bouvier, D.S., Fixemer, S., Heurtaux, T., Jeannelle, F., Frauenknecht, K.B. and Mittelbronn, M., 2022. The multifaceted neurotoxicity of astrocytes in ageing and age-related neurodegenerative diseases: a translational perspective. *Frontiers in Physiology*, *13*, p.814889. https://doi.org/10.3389/fphys.2022.814889

Brandl, C., Finger, R.P., Heid, I.M. and Mauschitz, M.M., 2023. Age-Related Macular Degeneration in an Ageing Society-Current Epidemiological Research. *Klinische Monatsblatter fur Augenheilkunde*, *240*(9), pp.1052-1059. https://doi.org/10.1055/a-2105-1064

Chen, Y., Yang, Z., He, X., Zhu, W., Wang, Y., Li, J., Han, Z., Wen, J., Liu, W., Yang, Y. and Zhang, K., 2023. Proanthocyanidins inhibited colorectal cancer stem cell characteristics through Wnt/β-catenin signaling. *Environmental Toxicology*, *38*(12), pp.2894-2903. https://doi.org/10.1002/tox.23924

Chung, K.W., Kim, D.H., Jung, H.J., Arulkumar, R., Chung, H.Y. and Yu, B.P., 2023. Chronic inflammation as an underlying mechanism of ageing and ageing-related diseases. *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, pp.31-44. https://doi.org/10.1007/978-3-031-26576-1_3

Deepashree, S., Shivanandappa, T. and Ramesh, S.R., 2022. Genetic repression of the antioxidant enzymes reduces the lifespan in Drosophila melanogaster. *Journal of Comparative Physiology B*, *192*(1), pp.1-13. https://doi.org/10.1007/s00360-021-01412-7

Deolankar, S.C., Najar, M.A., Ramesh, P., Kanichery, A., Kudva, A.K., Raghu, S.V. and Prasad, T.K., 2023. Discovery of molecular networks of neuroprotection conferred by brahmi extract in Aβ42-induced toxicity model of Drosophila melanogaster using a quantitative proteomic approach. *Molecular neurobiology*, *60*(1), pp.303-316. https://doi.org/10.1007/s12035-022-03066-0

dos Santos Cardoso, A., Santos, E.G.G., da Silva Lima, A., Temeyer, K.B., de Leon, A.A.P., Junior, L.M.C. and dos Santos Soares, A.M., 2020. Terpenes on Rhipicephalus (Boophilus) microplus: Acaricidal activity and acetylcholinesterase inhibition. *Veterinary parasitology*, *280*, p.109090. https://doi.org/10.1016/j.vetpar.2020.109090

Durmaz, L., Erturk, A., Akyüz, M., Polat Kose, L., Uc, E.M., Bingol, Z., Saglamtas, R., Alwasel, S. and Gulcin, İ., 2022. Screening of carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzyme inhibition effects and antioxidant activity of coumestrol. *Molecules*, *27*(10), p.3091. https://doi.org/10.3390/molecules27103091

Fagbemi, K.O., Aina, D.A., Adeoye-Isijola, M.O., Naidoo, K.K., Coopoosamy, R.M. and Olajuyigbe, O.O., 2022. Bioactive compounds, antibacterial and antioxidant activities of methanol extract of Tamarindus indica Linn. *Scientific reports*, *12*(1), p.9432. https://doi.org/10.1038/s41598-022-13716-x

Ferreira, J., Santos, S. and Pereira, H., 2020. In vitro screening for acetylcholinesterase inhibition and antioxidant activity of Quercus suber cork and corkback xtracts. *Evidence-Based Complementary and Alternative Medicine*, *2020*(1), p.3825629. https://doi.org/10.1155/2020/3825629

Forman, H.J. and Zhang, H., 2021. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*, *20*(9), pp.689-709. https://doi.org/10.1038/s41573-021-00233-1

Gómez-García, A. and Medina-Franco, J.L., 2022. Progress and impact of Latin American natural product databases. *Biomolecules*, *12*(9), p.1202. https://doi.org/10.3390/biom12091202

Graur, A., Sinclair, P., Schneeweis, A.K., Pak, D.T. and Kabbani, N., 2023. The human acetylcholinesterase C-terminal T30 peptide activates neuronal growth through alpha 7 nicotinic acetylcholine receptors and the mTOR pathway. *Scientific Reports*, *13*(1), p.11434. https://doi.org/10.1038/s41598-023-38637-1

Guo, C., Zhang, H., Guan, X. and Zhou, Z., 2019. The anti-aging potential of neohesperidin and its synergistic effects with other citrus flavonoids in extending chronological lifespan of saccharomyces cerevisiae BY4742. *Molecules*, *24*(22), p.4093. https://doi.org/10.3390/molecules24224093

Herath, H.D., Taki, A.C., Sleebs, B.E., Hofmann, A., Nguyen, N., Preston, S., Davis, R.A., Jabbar, A. and Gasser, R.B., 2021. Advances in the discovery and development of anthelmintics by harnessing natural product scaffolds. *Advances in Parasitology*, *111*, pp.203-251.

https://doi.org/10.1016/bs.apar.2020.10.002

He, J., Li, X., Yang, S., Li, Y., Lin, X., Xiu, M., Li, X. and Liu, Y., 2021. Gastrodin extends the lifespan and protects against neurodegeneration in the Drosophila PINK1 model of Parkinson's disease. *Food & Function*, *12*(17), pp.7816-7824.https:// doi.org/10.1039/d1fo00847a

Jaafaru, M.S., Abd Karim, N.A., Enas, M.E., Rollin, P., Mazzon, E. and Abdull Razis, A.F., 2018. Protective effect of glucosinolates hydrolytic products in neurodegenerative diseases (NDDs). *Nutrients*, *10*(5), p.580. https://doi.org/10.3390/ nu10050580

Jaafaru, M.S., Muhammad, S.A., Mohammed, Z.K., Aliyu, Y. and Razis, A.F.A., 2024. Proanthocyanidins supplemented diet alter anti-aging-markers and improved lifespan in Drosophila melanogaster model. *Beni-Suef University Journal of Basic and Applied Sciences*, *13*(1), p.11. https://doi.org/10.1186/s43088-024-00469-x

Jaafaru, M.S., Nordin, N., Shaari, K., Rosli, R. and Abdull Razis, A.F., 2018. Isothiocyanate from Moringa oleifera seeds mitigates hydrogen peroxide-induced cytotoxicity and preserved morphological features of human neuronal cells. *PLoS One*, *13*(5), p.e0196403. https://doi.org/10.1371/journal.pone.0196403

Jin, K., Wilson, K.A., Beck, J.N., Nelson, C.S., Brownridge III, G.W., Harrison, B.R., Djukovic, D., Raftery, D., Brem, R.B., Yu, S. and Drton, M., 2022. Correction: Genetic and metabolomic architecture of variation in diet restriction-mediated lifespan extension in Drosophila. *PLoS Genetics*, *18*(4), p.e1010199. https://doi.org/10.1371/journal.pgen.1010199

Kristiani, L. and Kim, Y., 2023. The interplay between oxidative stress and the nuclear lamina contributes to laminopathies and age-related diseases. *Cells*, *12*(9), p.1234.https://doi.org/10.3390/cells12091234

Korovesis, D., Rubio-Tomás, T. and Tavernarakis, N., 2023. Oxidative stress in age-related neurodegenerative diseases: An overview of recent tools and findings. *Antioxidants*, *12*(1), p.131.https://doi.org/10.3390/antiox12010131

Lista, S., Vergallo, A., Teipel, S.J., Lemercier, P., Giorgi, F.S., Gabelle, A., Garaci, F., Mercuri, N.B., Babiloni, C., Gaire, B.P. and Koronyo, Y., 2023. Determinants of approved acetylcholinesterase inhibitor response outcomes in Alzheimer's disease: relevance for precision medicine in neurodegenerative diseases. *Ageing research reviews*, *84*, p.101819.https://doi.org/10.1016/j.arr.2022.101819

Liu, J.K., 2022. Antiaging agents: Safe interventions to slow aging and healthy life span extension. *Natural Products and Bioprospecting*, *12*(1), p.18.https://doi. org/10.1007/s13659-022-00339-y

Luna, E.M., Freitas, T.S., Campina, F.F., Costa, M.S., Rocha, J.E., Cruz, R.P., Júnior, D.S., Silveira, Z.S., Macedo, N.S., Pinheiro, J.C.A. and Pereira-Júnior, F.N., 2021. Evaluation of phytochemical composition, toxicity in Drosophila melanogaster and effects on antibiotics modulation of Plathymenia reticulata Benth extract. *Toxicology Reports*, *8*, pp.732-739.https://doi.org/10.1016/j.toxrep.2021.03.020

Mishra, E. and Thakur, M.K., 2023. Mitophagy: A promising therapeutic target for neuroprotection during ageing and age-related diseases. *British Journal of Pharmacology*, *180*(12), pp.1542-1561. https://doi.org/10.1111/bph.16062

Mohammed, Z.K., Jaafaru, M.S., Ibrahim, J., Usman, A.S., Jafaru, A. and Abubakar, R.I., 2022. Gum exudates of Acacia senegal linn is an alternative binding agent in Drosophila melanogaster culture for laboratory maintenance of stocks. *Science World Journal*, *17*(2), pp.294-302.

Mołoń, M., Dampc, J., Kula-Maximenko, M., Zebrowski, J., Mołoń, A., Dobler, R., Durak, R. and Skoczowski, A., 2020. Effects of temperature on lifespan of Drosophila melanogaster from different genetic backgrounds: Links between metabolic rate and longevity. *Insects*, *11*(8), p.470.https://doi.org/10.3390/insects110 80470

Muflihah, Y.M., Gollavelli, G. and Ling, Y.C., 2021. Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. *Antioxidants*, *10*(10), p.1530.https://doi.org/10.3390/antiox1010 1530

Nascimento, L.A., Nascimento, É.C. and Martins, J.B., 2022. In silico study of tacrine and acetylcholine binding profile with human acetylcholinesterase: docking and electronic structure. *Journal of Molecular Modeling*, *28*(9), p.252. https:// doi.org/10.1007/s00894-022-05252-2

Nichols, C.D., Becnel, J. and Pandey, U.B., 2012. Methods to assay Drosophila behavior. *JoVE (Journal of Visualized Experiments)*, (61), p.e3795.https://doi.org/10.3791/3795

Potęga, A., 2022. Glutathione-mediated conjugation of anticancer drugs: an overview of reaction mechanisms and biological significance for drug detoxification and bioactivation. *Molecules*, *27*(16), p.5252. https://doi.org/10.3390/molecules27165252.

Prohaska, J.R., 1980. The glutathione peroxidase activity of glutathione S-transferases. *Biochimica et Biophysica Acta (BBA)-Enzymology*, *611*(1), pp.87-98.https://doi.org/10.1016/0005-2744(80)90045-5

Qiu, D., Song, S., Chen, N., Bian, Y., Yuan, C., Zhang, W., Duan, H. and Shi, Y., 2023. NQO1 alleviates renal fibrosis by inhibiting the TLR4/NF-κB and TGF-β/Smad signaling pathways in diabetic nephropathy. *Cellular Signalling*, *108*, p.110712.https://doi.org/10.1016/j.cellsig.2023.110712

Rouka, E., Gourgoulianni, N., Lüpold, S., Hatzoglou, C., Gourgoulianis, K.I. and Zarogiannis, S.G., 2022. Prediction and enrichment analyses of the Homo sapiens-Drosophila melanogaster COPD-related orthologs: potential for modeling of human COPD genomic responses with the fruit fly. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *322*(1), pp.R77-R82. https://doi.org/10.1152/ ajpregu.00092.2021

Sarkar, C., Quispe, C., Jamaddar, S., Hossain, R., Ray, P., Mondal, M., Mohamed, Z.A., Jaafaru, M.S., Salehi, B., Islam, M.T. and Razis, A.F.A., 2020. Therapeutic promises of ginkgolide A: A literature-based review. *Biomedicine & Pharmacotherapy*, *132*, p.110908. https://doi.org/10.1016/j.biopha.2020.110908

Saxena, M., Prabhu, S.V., Mohseen, M., Pal, A.K., Alarifi, S., Gautam, N. and Palanivel, H., 2022. [Retracted] Antidiabetic Effect of Tamarindus indica and Momordica charantia and Downregulation of TET-1 Gene Expression by Saroglitazar in Glucose Feed Adipocytes and Their Involvement in the Type 2 Diabetes-Associated Inflammation In Vitro. *BioMed Research International*, *2022*(1), p.9565136. https://doi.org/10. 1155/2022/9565136

Semaniuk, U.V., Gospodaryov, D.V., Strilbytska, O.M., Kucharska, A.Z., Sokół-Łętowska, A., Burdyliuk, N.I., Storey, K.B., Bayliak, M.M. and Lushchak, O., 2022. Chili-supplemented

food decreases glutathione-S-transferase activity in Drosophila melanogaster females without a change in other parameters of antioxidant system. *Redox Report*, *27*(1), pp.221-229. https://doi.org/10. 1080/13510002.2022.2123884

Sharma, R. and Diwan, B., 2023. Lipids and the hallmarks of aging: from pathology to interventions. *Mechanisms of Ageing and Development*, p.111858.https://doi.org/10.1016/j. mad.2023.111858

Siegel, D., Kepa, J.K. and Ross, D., 2007. Biochemical and genetic analysis of NAD (P) H: quinone oxidoreductase 1 (NQO1). *Current Protocols in Toxicology*, *32*(1), pp.4-22.https://doi.org/10.1002/0471140856.tx0422s32

Tallini, L.R., da Silva, C.R., Jung, T., Alves, E.D.O., Baldin, S.L., Apel, M., Timmers, L.F., Rico, E.P., Bastida, J. and Zuanazzi, J.A.S., 2023. Acetylcholinesterase inhibition activity of Hippeastrum papilio (Ravenna) Van Scheepen (Amaryllidaceae) using zebrafish brain homogenates. *Life*, *13*(8), p.1721. https://doi.org/10. 3390/life13081721

Taylor, P., Shyong, Y.J., Samskey, N., Ho, K.Y., Radic', Z., Fenical, W., Sharpless, K.B., Kovarik, Z. and Camacho-Hernandez, G.A., 2021. Ligand design for human acetylcholinesterase and nicotinic acetylcholine receptors, extending beyond the conventional and canonical. *Journal of neurochemistry*, *158*(6), pp.1217-1222. https://doi.org/10.1111/jnc.15335

Vágási, C.I., Vincze, O., Pătraș, L., Osváth, G., Pénzes, J., Haussmann, M.F., Barta, Z. and Pap, P.L., 2019. Longevity and life history coevolve with oxidative stress in birds. *Functional Ecology*, *33*(1), pp.152-161. https://doi.org/10.1111/1365-2435.13228

Vives-Bauza, C., Starkov, A. and Garcia-Arumi, E., 2007. Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase. *Methods in cell biology*, *80*, pp.379-393.https://doi.org/10. 1016/S0091-679X(06)80019-1

Wang, S., Xue, J., Zhang, S., Zheng, S., Xue, Y., Xu, D. and Zhang, X., 2020. Composition of peony petal fatty acids and flavonoids and their effect on Caenorhabditis elegans lifespan. *Plant Wang, T.H., Tseng, W.C., Leu, Y.L., Chen, C.Y., Lee, W.C., Chi, Y.C., Cheng, S.F., Lai, C.Y., Kuo, C.H., Yang, S.L. and Yang, S.H., 2022. The flavonoid corylin exhibits lifespan extension properties in mouse. Nature Communications, 13(1), p.1238.*https://doi.org/ 10.1038/s41467-022-28908-2

Warnsmann, V., Hainbuch, S. and Osiewacz, H.D., 2018. Quercetin-induced lifespan extension in Podospora anserina requires methylation of the flavonoid by the O-methyltransferase PaMTH1. *Frontiers in genetics*, *9*, p.160. https://doi.org/10.3389/fgene.2018.00160

Worek, F., Mast, U., Kiderlen, D., Diepold, C. and Eyer, P., 1999. Improved determination of acetylcholinesterase activity in human whole blood. *Clinica chimica acta*, *288*(1-2), pp.73-90. https://doi.org/10.1016/S0009-8981(99)00144-8

Xie Y, Deng Q, Guo M, Li X, Xian D, Zhong J., 2023. polyphenolics: a novel approach to Henoch–Schonlein purpura through balancing immu- nity and arresting oxidative stress via TLR4/MyD88/NF-kappaB signaling pathway (Review). Exp Ther Med 25:300. https://doi.org/10.3892/etm. 2023.11999

Yang, F., Xiu, M., Yang, S., Li, X., Tuo, W., Su, Y., He, J. and Liu, Y., 2021. Extension of drosophila lifespan by astragalus polysaccharide through a mechanism dependent on antioxidant and insulin/IGF-1 signaling. *Evidence-Based Complementary and Alternative Medicine*, *2021*(1), p.6686748.. https://doi.org/10.1155/2021/ 6686748

Yuan, R., Musters, C.J.M., Zhu, Y., Evans, T.R., Sun, Y., Chesler, E.J., Peters, L.L., Harrison, D.E. and Bartke, A., 2020. Genetic differences and longevity-related phenotypes influence lifespan and lifespan variation in a sex-specific manner in mice. *Aging Cell*, *19*(11), p.e13263. https://doi.org/10.1111/acel.13263

Zhang, G., Devo, P., O'Leary, V.B. and Ovsepian, S.V., 2023. Ageing perspective on cognitive outcomes from auxiliary reproductive hormone adjustments. *Heliyon*. https://doi.org/10.1016/j.heliyon.2023.e19050

Zhang, X., Song, X., Hu, X., Chen, F. and Ma, C., 2023. Health benefits of proanthocyanidins linking with gastrointestinal modulation: An updated review. *Food Chemistry*, *404*, p.134596. https://doi.org/10.1016/j.foodchem.2022.134596

Zheng, W.Q., Zhang, J.H., Li, Z.H., Liu, X., Zhang, Y., Huang, S., Li, J., Zhou, B., Eriani, G., Wang, E.D. and Zhou, X.L., 2023. Mammalian mitochondrial translation infidelity leads to oxidative stress–induced cell cycle arrest and cardiomyopathy. *Proceedings of the National Academy of Sciences*, *120*(37), p.e2309714120. https://doi.org/10.1073/pnas. 2309714120