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Original Article

Dietary Supplementation of Quercetin Delays High-Sugar Diet-Induced Premature Ovarian Aging in *Drosophila melanogaster*

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ABSTRACT

Background: High-Sugar Diets (HSD) constitute a significant lifestyle concern, contributing to metabolic disorders and accelerated aging. The fruit fly, *Drosophila melanogaster*, shares significant genetic and metabolic similarities with humans, making it an excellent model for studying diet-induced premature aging, particularly of the ovary. **Objectives:** This study aimed to investigate the potential of the dietary flavonoid quercetin, a compound abundant in plant sources, to alleviate HSD-induced premature ovarian aging in *D. melanogaster*. **Materials and Methods:** Female flies were fed a Control Diet (CD), HSD (30% sucrose), or HSD supplemented with various quercetin doses (0.05-0.5 mg/g) for 20 days. Survival, climbing ability, ovarian morphology, and biochemical markers (glucose, triglycerides, oxidative stress, acetylcholinesterase activity) were assessed. Gene expression related to oxidative stress and hormonal signaling was analysed via RT-qPCR. **Results:** Quercetin at 0.1 and 0.25 mg/g significantly extended lifespan, improved climbing performance, and restored ovarian architecture in HSD-fed flies. It normalized metabolic profiles (glucose, TAG, protein levels), reduced oxidative stress (lipid peroxidation), and modulated acetylcholinesterase activity. Quercetin also upregulated key genes involved in antioxidant defense (Nrf2, FOXO) and steroid hormone signaling (ECR, JHAMT, Kr-h1), which HSD disrupted. **Conclusion:** Quercetin supplementation effectively delays HSD-induced premature ovarian aging in *Drosophila* by mitigating metabolic dysfunction, oxidative stress, and hormonal imbalance, highlighting its potential as a protective dietary intervention.

Keywords: Metabolic disorders, High sugar diet, Ovarian aging, Phytochemicals, Quercetin.

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INTRODUCTION

In recent decades, diet-related diseases have increased exponentially. Consuming a calorie surplus and a carbohydrate-rich diet is a primary concern in modern society. High sugar intake disrupts metabolic homeostasis and increases susceptibility to infection (Darby *et al*., 2023). *Drosophila melanogaster* is an excellent model organism due to its genetic tractability and ability to share similar organs or tissues that regulate metabolic homeostasis and the female reproductive system with humans. Hence, many study groups have already employed flies to investigate the effects of various factors, such as nutritional intake, on their survival, locomotor efficiency, Enzyme activities, metabolism, behavior, aging, and especially reproductive health. The high conservation of metabolic tissues and pathways, advanced metabolic experimentation techniques, ease of dietary manipulation, and short life cycles have made it a significant model in metabolic disease research (Tennessen *et al*., 2014).

The ovaries are one of the endocrine organs. The ovary plays a crucial role in female fertility by regulating growth, follicular maturation, and the reproductive hormonal cycle. Reducing the quantity and quality of the follicular reserve, characterized by a decrease in size and an increase in age, is a hallmark of ovarian aging (Díaz-Hernández *et al*., 2022). Recent research has uncovered the links between ovarian and systemic aging (Alam *et*

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al., 2014). Decreased ovarian activity, characterized by increased levels of Follicle-Stimulating Hormone (FSH) and decreased levels of Estradiol (E2), in women under the age of 40 years is defined as Premature Ovarian Failure (POF). Hypercaloric diets contribute to aging with metabolic disorders and can cause damage to ovarian health, leading to premature aging (Díaz-Hernández *et al.*, 2022).

A High-Sucrose Diet (HSD) leads to deteriorated ovarian health and accelerates aging when exposed early. A study described that the length and diameter of older ovaries were shortened and atrophied in females fed a high-sucrose diet. Atrophy of the ovary results in a decrease in steroid hormone secretion. A study showed that HSD could impair reproductive health by reducing the number of eggs produced through early deaths of germline cysts and vitellogenic egg degeneration, confirming the effect of HSD but not obesity (Nunes & Drummond-Barbosa, 2023). Oxidative stress is a significant factor in the fertility reduction in women. Hence, *Drosophila* provides a convenient method for studying egg development under various environmental and genetic conditions, offering insights into developmental and reproductive biology.

Phytochemicals, also known as botanicals, have been found to possess anti-aging properties. Among these, quercetin is one of the most abundant dietary flavonoids found in citrus fruits, broccoli, green leafy vegetables, buckwheat, nuts, olives, onions, apples, green tea, red grapes, and berries. Quercetin is known for its wide range of biological activities, including antioxidant, anti-inflammatory, anti-obesity, antimicrobial, antidiabetic, antiviral, and anticancer properties. Recently, quercetin has been explored for its potential to prolong the lifespan of various organisms, including zooplankton, yeast, *Caenorhabditis elegans, Drosophila*, and mice (D. Yang *et al.*, 2020). Additionally, quercetin helps reduce oxidative stress caused by hyperglycemia by managing blood glucose and lipid levels in individuals with diabetes mellitus. It ameliorates insulin resistance and can enhance healthy ovarian follicle development, restoring the healthy anatomy of the ovary, comparable to the metformin group (Neisy *et al.*, 2019). Recent studies suggest that quercetin may influence ovarian development and mimic estrogenic effects by directly binding to estrogen receptors, potentially aiding in the management of conditions related to estrogen imbalance (Wang *et al.*, 2022). Quercetin can prevent various female reproductive disorders by enhancing ovarian folliculogenesis and oogenesis, improving oocyte and embryo quality, and boosting fecundity in different species (Sirotkin, 2023). Quercetin supplementation is beneficial in the treatment of Polycystic Ovary Syndrome (PCOS) and ovarian cancer. Quercetin protects the ovaries from aging by reducing oxidative stress, enhancing the antioxidant capacity of the ovary by upregulating specific oxidative stress-related genes, and improving ovarian function. However, the overall information about the role of quercetin in delaying ovarian aging is limited.

In the given study, we are trying to investigate the defensive role of quercetin in *Drosophila melanogaster* against premature aging induced by a high-sugar diet that causes significant ovarian damage by checking the overall metabolic profile, including total protein content, weight, TAG, survival, antioxidant systems, enzyme assays, and its transcriptomic analysis.

MATERIALS AND METHODS

Fly stocks and culture requirements

A stock of *Drosophila melanogaster* Oregon R virgin females was used for all bio-assays to rule out the possibility that egg-laying contributes to lifespan. The flies were crossed and cultured on the standardized food medium at 25°C. We raised the larvae to the pupal stage on a standard Control Diet (CD) containing 5% (w/w) sucrose. Next, the newly emerged adult flies were divided into two treatment groups: CD and HSD. The newly emerged female flies were segregated and reared on either the Control Diet (CD) or the High-Sugar Diet (HSD). We prepared the media by adding the desired amount of sucrose to the CD, resulting in a 30% (w/v) diet (Witek *et al.*, 2022).

Quercetin dose standardization

Quercetin was purchased from HIMEDIA Laboratories in India. Initial standardization was conducted using known concentrations of quercetin, specifically 0.05-0.5 mg/g. Based on the results of quercetin treatment alone, we selected 0.05 mg/g, 0.1 mg/g, 0.25 mg/g, and 0.5 mg/g for the subsequent treatments. Subsequently, concentrations of 0.1-0.25 mg/g were utilized based on the standardization results. While using quercetin treatment, the control diet and HSD were supplemented with concentrations of 0.05, 0.1, 0.25, and 0.5 mg/g of quercetin by dissolving it in ethanol. Mortality was monitored daily. Climbing ability and ovarian damage were assessed every fifth day of treatment, and a 20-day window period was established for additional assays. Then, we also investigated the impact of HSD on the lifespan of female *D. melanogaster*, which was up to 20 days.

Weight estimation

After 20 days of treatment with their respective diets, 10 female flies were weighed to determine their total weight. Each group was studied with at least five replicates.

Climbing assays

The climbing assay assessed the flies' locomotor performance. The assay was carried out by taking surviving flies into the sterilized tube with dimensions of 11 cm in length and 3.5 cm in diameter, with a 6 cm mark (Adedara *et al.*, 2016). Usually, flies will fly to the top of the tube, whereas the flies with locomotor defects struggle to reach the same height. The locomotor efficiency was

calculated by determining the average number of flies crossing the designated 6 cm mark in 30 sec from the base among all the flies tested.

Ovarian dimensions

Ovaries of the control and treated flies were dissected in the buffer to investigate the impact of Quercetin treatment on the overall morphology of ovaries post-treatment; ovaries were dissected in a buffer at room temperature. Specifically, 15-20 adult flies were anesthetized by keeping them on ice, and their ovaries were dissected. A stereomicroscope was used to measure the length and diameter of each ovary dissected.

Biochemical analysis

Preparation of tissue homogenates

Flies were anesthetized on ice and homogenized in Microcentrifuge tubes with 0.1 M phosphate buffer at pH 7.4. The homogenates were centrifuged for 10 min at 10,000 rpm at 4°C. The supernatant was then used for further biochemical analysis.

Determination of total protein content

The total protein content of flies was determined by the Bradford assay. Bovine Serum Albumin (BSA) was used as a standard, and protein normalization was performed at a concentration of 1mg/mL for subsequent assays.

Glucose and Triacylglyceride (TAG) estimation

Glucose and triacylglyceride levels were determined using standard diagnostic kits, namely Liquick Cor-GLUCOSE and Liquick Cor-TG (PZ Cormay S.A., Łomianki, Poland). For the Glucose assay, the test samples were homogenized in 50 mM KPi buffer (1:10, w/v). For the TAG assay, we used 10 mM phosphate buffered saline (PBST) with 0.05% Triton X-100) (1:50, w/v) for homogenization. The homogenates were first heated at 70°C for 10 min, then cooled, and afterward used for the assays. Calibration curves were generated using standards of glucose (2-20 µg/mL) and TAG (3-30 µg/mL). The absorbance was taken at 530 nm and 520 nm for glucose and TAG, respectively.

Evaluation of oxidative stress markers

Determination of Superoxide Dismutase activity (SOD)

SOD activity was determined based on the percent inhibition of formazon formation. The enzyme activity was expressed as units/mg of protein by reading absorbance at 560 nm.

Estimation of Reactive Oxygen Species (ROS)

A Nitro Blue Tetrazolium (NBT) assay was used to estimate the total reactive oxygen species in the samples. In this assay, the yellow water-soluble powder is reduced to blue insoluble formazan crystals. Samples were incubated together with NBT for 1 hour, and the reaction was stopped by adding 50% acetic acid (150 µL) and thorough vortexing. Absorbance was measured at 595 nm (Hyung *et al.*, 2006).

Lipid Peroxidation (MDA) Assay

The reaction mixture contains 50 µL of tissue homogenate in a mixture of 150 µL of 8.1% Sodium Dodecyl Sulfate (SDS), 250 µL of acetic acid solution (in 2.5 M HCl, pH 3.4), and 250 µL of 0.8% Thiobarbituric Acid (TBA). The mixture was then incubated at 100°C for 1 hr. A 532 nm wavelength was used to quantify the Thiobarbituric Acid Reactive Substances (TBARS) formed in the reaction, as measured using a microtiter plate reader, and calculated as MDA (Ohkawa *et al.*, 1979).

Acetylcholinesterase (AChE) activity assay

Acetylcholinesterase (AChE) activity was calculated using the protocol described by Ellman *et al.*, (1959) and Ellman *et al.*, (1961). 15 µL of tissue homogenate from 50 treated fly heads was taken in 50 µL of 0.1 M potassium phosphate buffer (pH 7.4) with 50 µL of distilled water. Then, 30 µL of 8 mM substrate (acetylthiocholine iodide) was added. 30 µL of 10 mM 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB) served as the dye. The ChE activity was calculated and expressed as µmol AChE/ µg protein after reading at 412 nm.

RT-qPCR

HSD alone or with co-treatment of quercetin was given to the flies' ovaries for 7 days. Later, it is weighed and stored using liquid nitrogen. The Magnetic Tissue/Cell/Blood Total RNA Kit was used to isolate the total RNA from the samples. After isolating the RNA using the script cDNA synthesis kit, 1 µg of RNA was reverse-transcribed into cDNA. RT-PCR was performed in a thermal cycler using the following cycling conditions: 40 cycles of 95°C for 5 min, 95°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec. The β-actin gene served as the control, and genes involved in the steroid signalling pathway were selected. The primer sequences are provided in Supplementary Table S1. Gene expression levels were determined using the threshold Cycle (Ct) method, and the expression levels of each group were normalized to those of the control group.

Statistical Analysis

The data of the experiment were represented with mean ± SE. Experiments were conducted three times in triplicate. Statistical analysis was performed using one-way ANOVA using IBM SPSS software version 26. Multiple comparisons were made using Tukey's *post hoc* analysis, and the significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, with $^{ns}p > 0.05$ compared to the control. Fly survival was analyzed using the Kaplan-Meier method, and the significance of the log-rank test was assessed.

RESULTS

Effect of Quercetin on survival, Ovarian morphology, weight, and climbing

Supplementary Figure 1 shows that the overall lifespan of the flies was shortened significantly between the (CD) and HSD groups (p<0.0001). The highest dose (0.5 mg/g) showed a similar shortened lifespan as HSD. The quercetin concentrations of 0.1 mg/g and 0.25 mg/g showed the best results compared to 0.05 mg/g and 0.5 mg/g in HSD co-treatment, which demonstrated an extended lifespan (p = 0.0134). The 0.25 mg/g dose showed a longer lifespan compared to the control group. These results suggest that treatment with 0.1 mg/g and 0.25 mg/g of quercetin contributed to lifespan extension, even under HSD conditions. Therefore, we selected these two Quercetin concentrations for further experiments.

Female flies were administered varying concentrations for over 20 days to assess their potential in reversing High-Sugar Diet (HSD)-induced premature ovarian aging. Quercetin at 0.1 mg/g and 0.25 mg/g demonstrated the most significant protective effects among the tested doses. The control group, which maintained a regular diet, also showed improvements. The morphological analysis of the ovaries, as illustrated in Supplementary Figure 2, revealed improved ovarian structure and follicular integrity in flies treated with these optimal doses, suggesting a dose-dependent protective effect of quercetin against metabolic stress-induced reproductive decline.

Accordingly, we weighed the flies 20 days after the end of the HSD treatment (Supplementary Figure 3). The flies showed a slight reduction in body weight on the HSD diet, but it was not statistically significant compared to CD. HSD flies demonstrated reduced body weight in comparison to CD flies. However, after

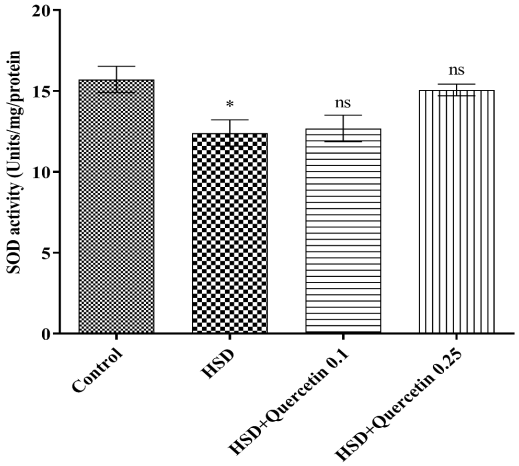

Figure 1a: Effects of Quercetin on SOD activity in HSD-fed *Drosophila* *p<0.05, **p<0.01, ***p<0.001 compared with control.

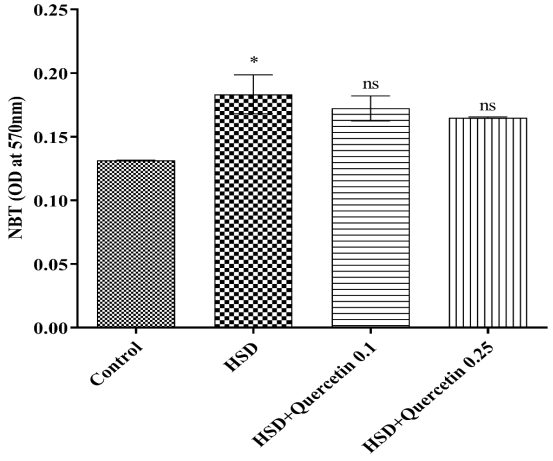

Figure 1b: Effects of Quercetin on ROS levels in HSD-fed *Drosophila* *p<0.05, **p<0.01, ***p<0.001 compared with control.

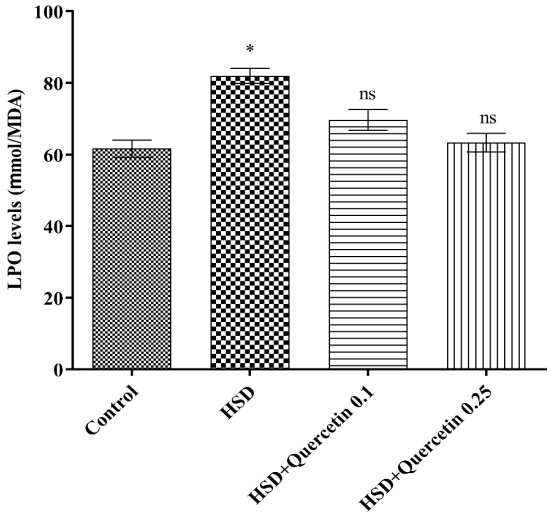

Figure 1c: Effects of Quercetin on LPO levels activity in HSD-fed *Drosophila**p<0.05, **p<0.01, ***p<0.001 compared with control.

treatment with quercetin at 0.1 mg/g and 0.25 mg/g, their weight was comparable to that of CD. However, the highest dose of 0.5 mg/g Quercetin and HSD showed significant weight reduction.

When locomotor ability was investigated, female flies exhibited improved climbing performance when treated with varying concentrations of quercetin, with 0.1 mg/g and 0.25 mg/g yielding the most favourable outcomes (Supplementary Figure 4).

Effect of Quercetin on biochemical parameters

The protein concentration was decreased in the HSD-fed flies compared to the controls. It was restored after co-treatment with Quercetin (Supplementary Figure 5a). After feeding flies with a High-Sugar Diet (HSD), there was an increase in glucose levels. A significant rise was noted in Triacylglycerol (TAG) levels compared to the control group which indicates that the flies store excess glucose in the form of TAG (Supplementary Figure 5b, 5c).

Quercetin alleviates HSD oxidative stress

We focused our investigation on several oxidative stress markers in flies. No difference was observed in SOD and NBT activity after HSD treatment compared to the control group (Figures 1a, 1b). However, co-treatment with 0.1 mg/g of quercetin resulted in a significant decrease in NO and SOD levels ($p < 0.05$). Meanwhile, LPO values were disrupted in flies fed on HSD. MDA, or malondialdehyde, is a byproduct of lipid peroxidation that indicates oxidative stress levels (Figure 1c). This demonstrates that the MDA level in the treatment group was significantly higher than in the control group, indicating a 33% increase ($p < 0.05$). Conversely, the group receiving 0.1 mg/g had MDA levels that were 13% lower than those in the High-Sugar Diet (HSD) group ($p < 0.05$). Notably, the group treated with 0.25 mg/g completely restored MDA levels to those in the control group. There was no difference among the groups in terms of other oxidative stress parameters studied.

Quercetin altered the AChE activity

The sucrose intake at this concentration caused an increase of approximately 41.7 % (3.936 U/µg of protein) in AChE activity than the control group (Figure 2). The changes caused by HSD were reversed by 0.1 mg/g and 0.25 mg/g doses by 13.3% (2.967 U/µg of protein) and 8.3% (3.2458 U/µg of protein) respectively ($p<0.01$).

Effect of Quercetin on oxidative stress, inflammatory, and hormonal genes

We measured the expression of genes related to steroid signalling pathways in hyperglycemic female *Drosophila* by triggering steroid signals. The ECR expression levels, which code for the ecdysone receptor, and early transcription factors such as E74 and Br were reduced in the hyperglycemic group compared to the control group. However, significant improvement was observed in the treatment with Quercetin (Figure 3a, Figure 3b). ECR (Figure 3c), early transcription factor Br, and Kr-h1 (Figure 2d) protein expression levels were reduced in the HSD group compared to the control group. However, all were significantly increased with 0.25 mg of Quercetin treatment, aligning with mRNA expression results. We found a reduction in the expression levels of Juvenile Hormone Acid O-methyltransferase (JHAMT) (Figure 3e) and hydroxymethylglutaryl-CoA reductase (HMGR) (Figure 3f) in the hyperglycemic group compared to the control group. However, these levels increased after Quercetin administration.

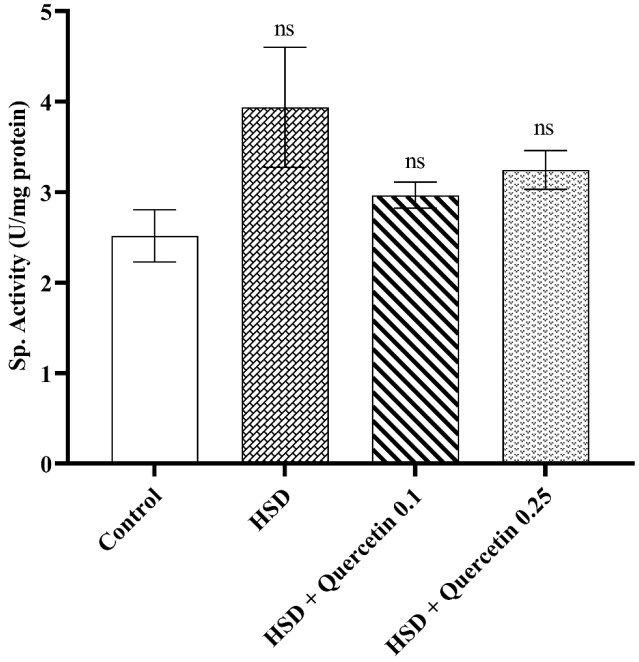

Figure 2: Effects of Quercetin on AChE activity in HSD-fed *Drosophila* *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with control.

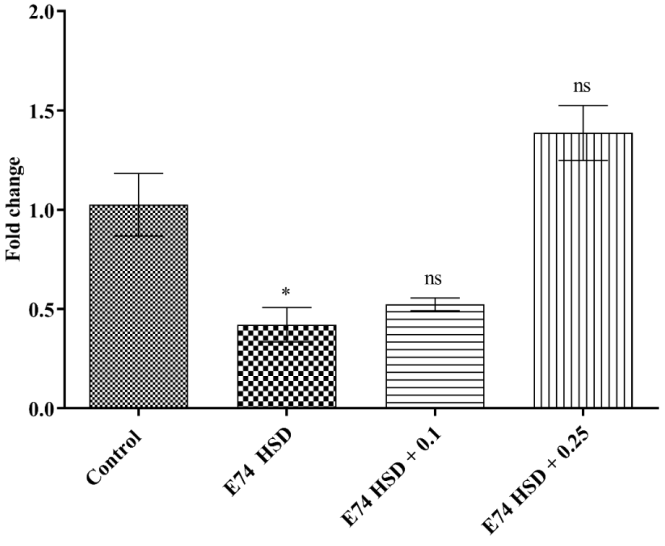

Figure 3a

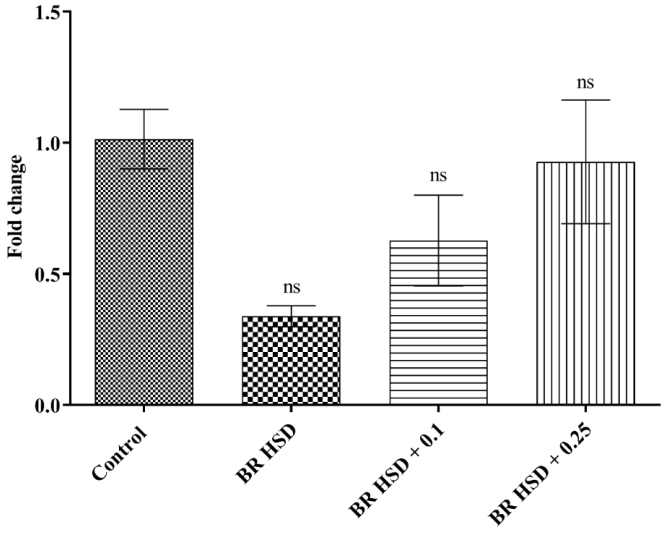

Figure 3b

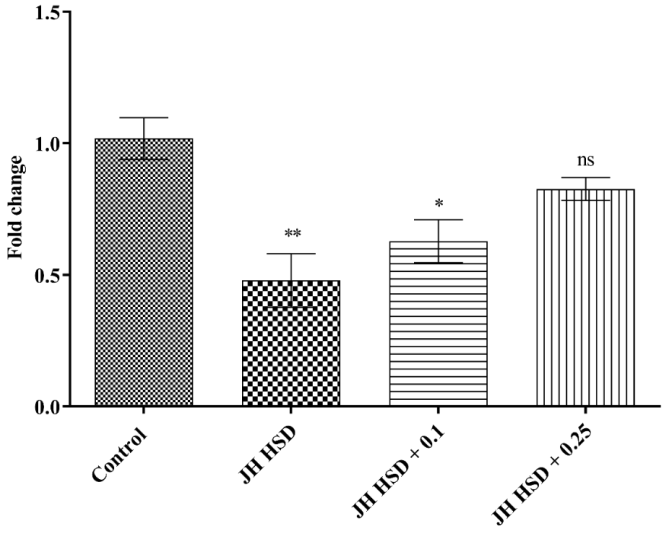

Figure 3e

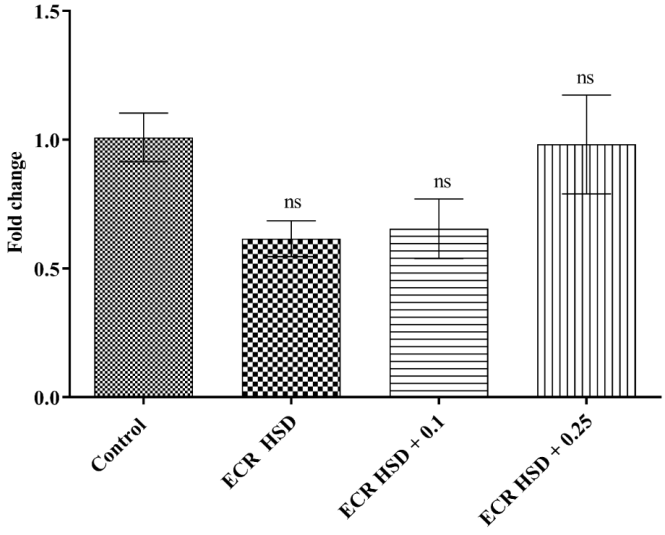

Figure 3c

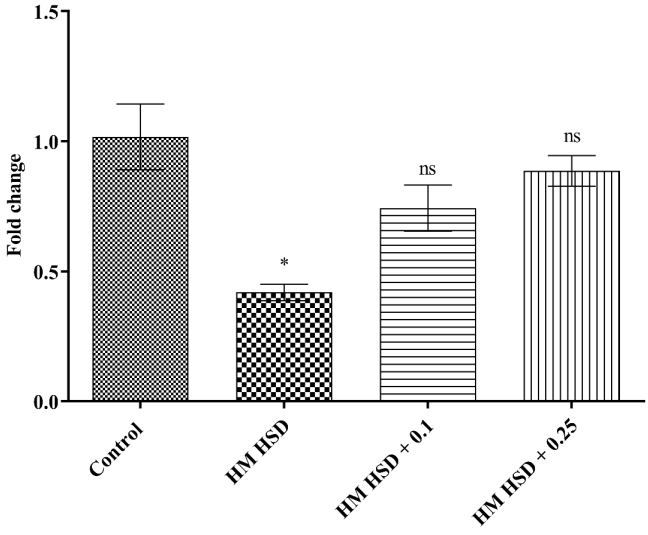

Figure 3f

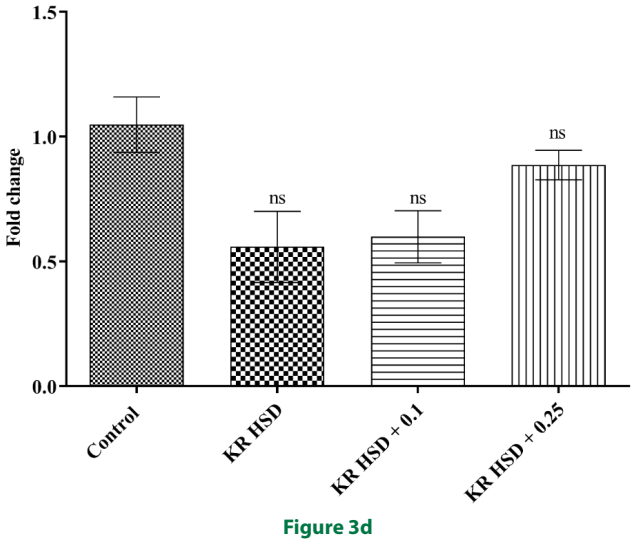

Figure 3d

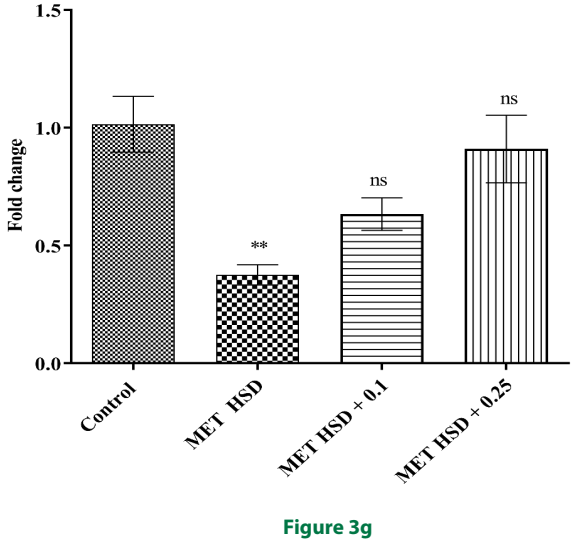

Figure 3g

Figure 3: Effect of Quercetin on expression of steroidal signal-related genes in *Drosophila*. (a) E74; (b) Br; (c) ECR; (d) kr-h1; (e) JHAMT; (f) HMGR; (g) Met; Relative expression levels of beta-actin were used as loading control. **p*<0.05, ***p*<0.01, ****p*<0.001 compared with control.

At a dose of 0.25 mg, the levels of Krüppel Homolog 1 (Kr-h1) and Methoprene Tolerant (Met) increased (Figure 3g).

Oxidative stress link

Our results showed that Nrf2 expression in the HSD group was reduced significantly in a dose-dependent manner (Figure 4a). HSD inhibited the activity of the FOXO gene in the ovary (Figure 4b). At the same time, quercetin enhanced this gene's activity in a dose-dependent manner. Following this, we also found downregulation of FOXO and p38 genes (Figure 4c), which are typically upregulated by quercetin. We found elevated expression of MAPK in the HSD group (Figure 4d), which quercetin normalized in a dose-dependent way.

DISCUSSION

High-sugar diets play a significant role in inducing hyperglycemia in *D. melanogaster*. Hyperglycemia accompanied by weight loss is considered an indicator of insulin signaling failure. In

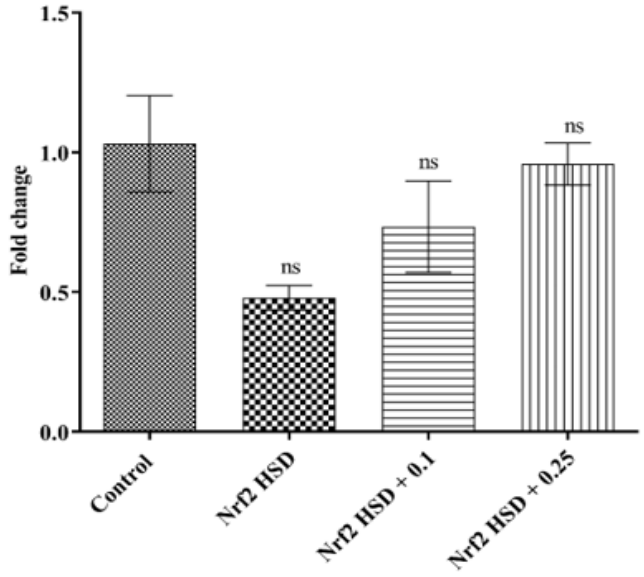

Figure 4a.

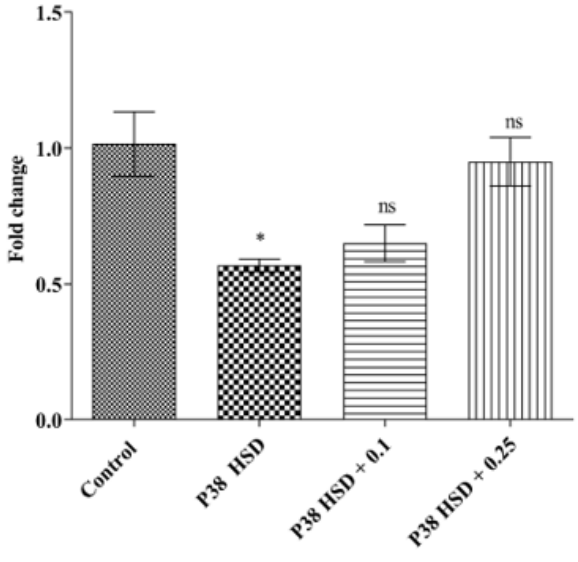

Figure 4c.

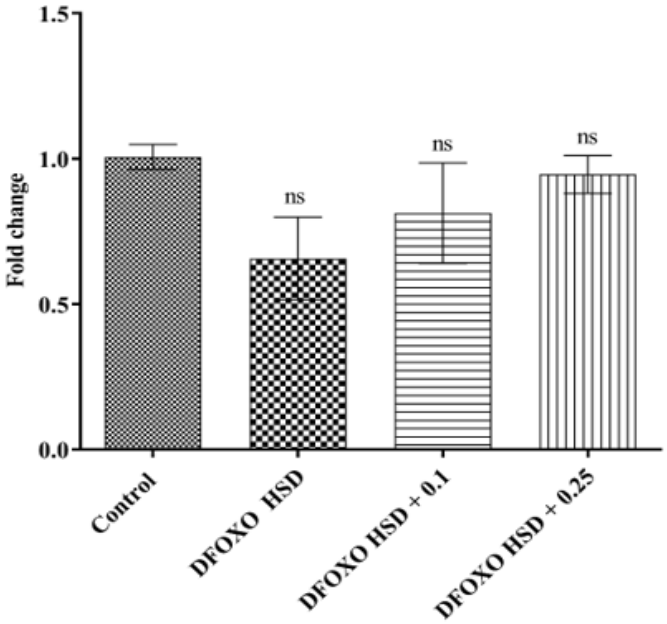

Figure 4b.

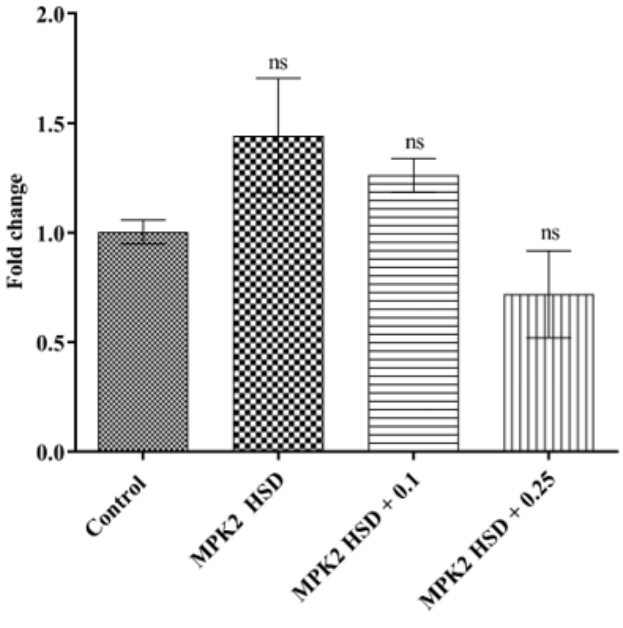

Figure 4d.

Figure 4: Effect of Quercetin on expression of oxidative stress-related genes in *Drosophila*. (a) Nrf2; (b) DFOXO; (c) p38; (d) MPK2; Relative expression levels of beta-actin were used as loading control. **p*<0.05, ***p*<0.01, ****p*<0.001 compared with control.

comparison to CD flies, HSD flies demonstrated reduced body weight (J. Yang *et al.*, 2023). Contrary to what some researchers observed, diet-induced insulin resistance and weight gain in adult flies were observed when they were exposed to a high-sugar diet (van Dam *et al.*, 2020). This divergence presents a valuable opportunity to explore potential interventions that may mitigate the adverse effects of an unhealthy diet. One such potential intervention is the polyphenol compound quercetin. Various animal models, including mice and rats, have demonstrated the anti-obesity properties of quercetin. However, we have not extensively explored its effects on weight regulation in *Drosophila* under a high-sugar diet. When wild-type *Drosophila* were fed a high-sugar diet, it led to obesity and insulin resistance. Associated metabolic disorders, such as glycation, hyperglycemia, and insulin resistance, typically contribute to the reduced lifespan of adults on an HSD (Skorupa *et al.*, 2008). Thus, our model sheds light on the pathophysiology of high-sugar diets, which is not related to obesity, consistent with previously reported results (van Dam *et al.*, 2020). Researchers have extensively used the assessment of climbing proficiency to determine the changes in physiology due to nutritional interventions, including fitness and overall behaviour of flies. Prior research has shown that this indicator gradually decreases in flies as they age normally (Jones & Grotewiel, 2011). However, studies (Baenas & Wagner, 2022) have demonstrated its diminishment upon exposure to HSD. *Solanum anguivi* Lam. Fruit (Nakitto *et al.*, 2021), Avens's root extract (Günther *et al.*, 2021), and bayberry leaf proanthocyanidins (Wang *et al.*, 2022) significantly reduced glucose and triglyceride levels when fed with HSD in w1118 flies subjected to HSD supplementation. Quercetin protects against hyperglycemia-related oxidative stress; a high-sucrose diet is associated with a hyperglycemic state, which can lead to oxidative events (Folmer *et al.*, 2004). The elevated production of lipid peroxide groups indicates an enhanced level of lipid oxidation. These results indicated substantial oxidative stress in the HSD group with increased lipid peroxidation, whereas quercetin significantly reduced its level. (Ecker *et al.*, 2017) reported that flies developed from larvae fed on 30% HSD marked a decrease in AChE activity. *Syzigium cumini* restored its activity, contrary to our results. Rats fed a fructose-rich diet exhibited increased Acetylcholinesterase (AChE) activity, which aligns with our findings (Spagnuolo *et al.*, 2023). This increased AChE activity may be a mechanism by which cells adapt to prevent Acetylcholine (ACh) signaling from lasting too long.

Kr-h1 and dFOXO may participate in the transcriptional co-regulation. This can help understand the broad mechanism of maintaining metabolic homeostasis and coordinating organism growth, in which Kruppel-like factors integrate with insulin signaling. Transcription of Kr-h1 can be induced strongly by JH via its receptor Methoprene-tolerant (Met) (Kayukawa *et al.*, 2016). FOXO is crucial in facilitating the interaction between insulin signaling and other hormones of the insect system, such as Juvenile Hormone (JH) and ecdysteroids, eventually coordinating insect growth, overall development, and balancing metabolic processes

The increased expression of the genes JHAMT and HMGR promotes the biosynthesis of Juvenile Hormone (JH), as observed with the increased expression of the JH gene, as reported by Song & Zhou 2020. Signaling of Juvenile Hormone (JH), through its receptor Methoprene-tolerant (Met), regulates metamorphosis and reproduction in insects. JH signaling may directly regulate the maintenance of ovarian Germline Stem Cells (GSCs). The knockdown of Met and Gce, or Kr-h1, by RNA interference (RNAi) targeted to ovarian cap cells, resulted in a decline in the number of GSCs, indicating that signaling of JH/Met/Kr-h1 also functions in oogenesis in *D. melanogaster* (Luo *et al.*, 2020). RNAi-induced Knockdown of ovarian Met or Kr-h1 in the adult fat body resulted in a significant reduction in fecundity, characterized by decreased oviposition, increased overall ovary size, and the accumulation of mature eggs in the ovary, accompanied by a shortening of egg length (Luo *et al.*, 2021). These findings suggest that quercetin activates steroid signaling and enhances ovarian function in hyperglycaemic female *Drosophila*. Redox homeostasis is primarily regulated by the transcription factor Nrf2. The Nrf2 gene is essential for activating antioxidants in response to reactive oxygen species and protecting cells from damage (Ma, 2013). Signaling pathways that regulate stress and redox status influence FOXO and p38 proteins, impacting insulin signaling and diabetes (Kops *et al.*, 2002). FOXO plays a crucial role in regulating longevity, affecting ovarian function, and leading to reduced fertility. High sugar consumption impacts the function of the FOXO gene in *Drosophila* ovaries, potentially decreasing reproductive success by restricting nutrient availability (Dobson *et al.*, 2017). High blood sugar levels activate the crucial regulator of oxidative stress tolerance called the p38 Mitogen-Activated Protein Kinase (MAPK) pathway (Hou *et al.*, 2008). Similarly, the mRNA expression of Mitogen-Activated Protein Kinase (MAPK) decreased due to the presence of bayberry leaf proanthocyanidins (Wang *et al.*, 2022). Activated MAPK pathways can increase ROS production, possibly leading to cell oxidative stress (Jubaidi *et al.*, 2022). In an HSD, MAPK signaling pathways are frequently activated, leading to complications such as insulin resistance, inflammation, and increased oxidative stress. A significant aspect of the p38 pathway in high-sugar diets is its role in managing oxidative stress, which can be exacerbated by excessive sugar intake; loss of p38Kb can result in a shorter lifespan and age-dependent defects in locomotor behavior. Furthermore, p38Kb regulates oxidative stress (Ryan *et al.*, 2021).

CONCLUSION

The study demonstrated that quercetin significantly extended the lifespan of the tested *D. melanogaster* flies when supplemented on a high-sugar diet. It also showed enhanced climbing performance and a reduction in oxidative stress. Notably, the highest dose of

quercetin did not yield the same positive effects. Additionally, the research examined the impact of quercetin on premature ovarian aging. Flies on a high-sugar diet that were given quercetin showed improved climbing abilities, longer lifespans, and better ovarian structure than those on the sugar diet alone. This treatment appears to help manage oxidative stress and hormonal imbalances in the ovaries.

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ABBREVIATIONS

FSH: Follicle-Stimulating Hormone; **E2:** Estradiol; **POF:** Premature Ovarian Aging/Failure; **POI:** Premature Ovarian Insufficiency; **HSD:** High Sugar Diet; **ROS:** Reactive Oxygen Species; **PCOS:** Polycystic Ovary Syndrome; **CD:** Control Diet; **BSA:** Bovine Serum Albumin; **TAG:** Triacylglycerides; **KPi:** Potassium Phosphate; **PBST:** Phosphate-Buffered Saline with 0.05% Triton X-100; **SOD:** Superoxide Dismutase; **NBT:** Nitro Blue Tetrazolium; **SDS:** Sodium Dodecyl Sulfate; **TBA:** Thiobarbituric Acid; **TBARS:** Thiobarbituric Acid Reactive Substance; **MDA:** Malondialdehyde; **AChE:** Acetylcholine Esterase; **DTNB:** 5,5'-Dithiobis (2-nitrobenzoic) Acid; RT-qPCR**:** Reverse Transcriptase Quantitative Polymerase Chain Reaction; **LPO:** Lipid Peroxidation; **ECR:** Ecdysone Receptor Expression; **Nrf2:** Nuclear Factor Erythroid 2-Related Factor 2; **FOXO:** Forkhead Box O; **JH:** Juvenile Hormone; **Met:** Methoprene Tolerant; **Kr-h1:** Krüppel Homolog 1; **JHAMT:** Juvenile Hormone Acid O-Methyltransferase; **HMGR:** Hydroxyl-Methylglutaryl-CoA Reductase; **GSCs:** Germline Stem Cells; **RNAi:** RNA Interference; **GCE:** Germline Stem Cell Gene; **MAPK:** Mitogen-Activated Protein Kinase; **Ct:** Threshold Cycle.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

SM: Methodology and Investigation. AVV: Formal Analysis, Writing - original draft, VJ: Methodology and Investigation, RM: Methodology, BK: Writing, review & editing, PK: Writing, review & editing, SP: Writing, review & editing, DK: Writing, review & editing. AS: Conceptualization, Methodology, Investigation, Supervision, Administration, Resources, Formal Analysis, writing - original draft.

SUMMARY

The quercetin a phytochemical was used to check its anti-aging properties induced by HSD. When HSD flies were administered quercetin treatment, they showed an improved lifespan, a life span, protective effect on ovarian morphology and recovered locomotor activity. The quercetin treatment decreased MDA levels, AChE activity in the co-treated HSD group and overall oxidative stress by upregulating the FOXO and p38 genes. So according to our study quercetin can be the good candidate for combating premature ovarian aging caused by HSD.

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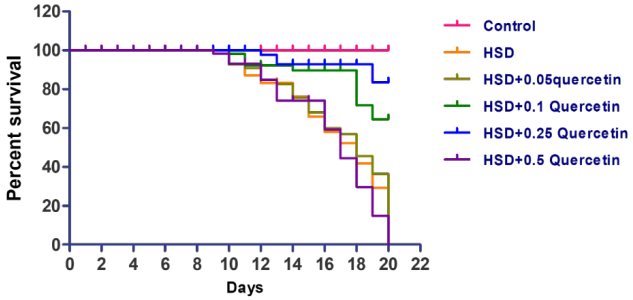

Figure S1: Effects of various concentrations of Quercetin on HSD-fed *Drosophila* Percent survival.

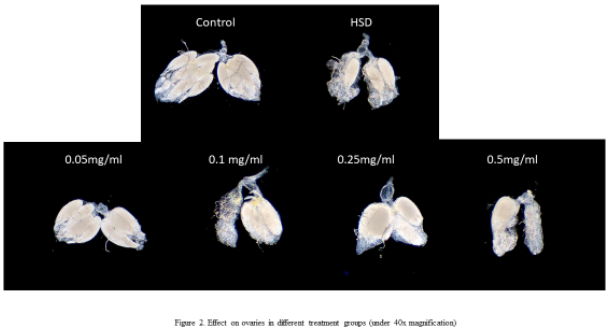

Figure 2. Effect on ovaries in different treatment groups (under 40x magnification)

Figure S2: Effect on ovaries in different treatment groups (under 40x magnification).

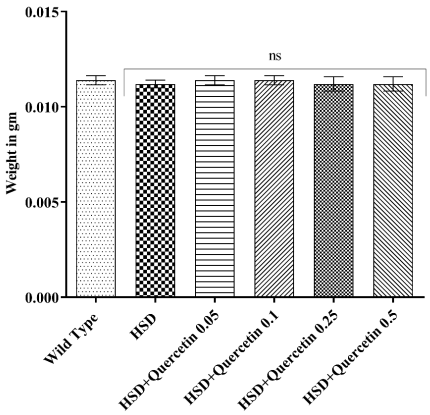

Figure S3: Effects of various concentrations of Quercetin on HSD-fed *Drosophila* weight *p<0.05, **p<0.01, ***p<0.001 compared with control.

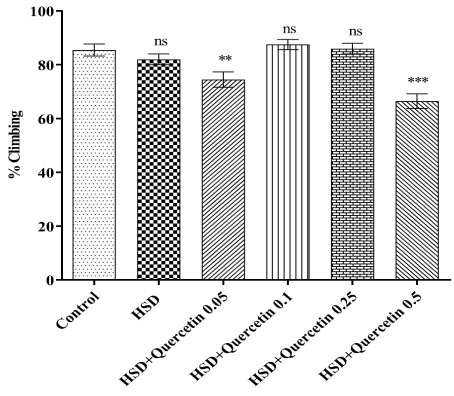

Figure S4: Effects of various concentrations of Quercetin on HSD-fed *Drosophila* Climbing ability *p<0.05, **p<0.01, ***p<0.001 compared with control.

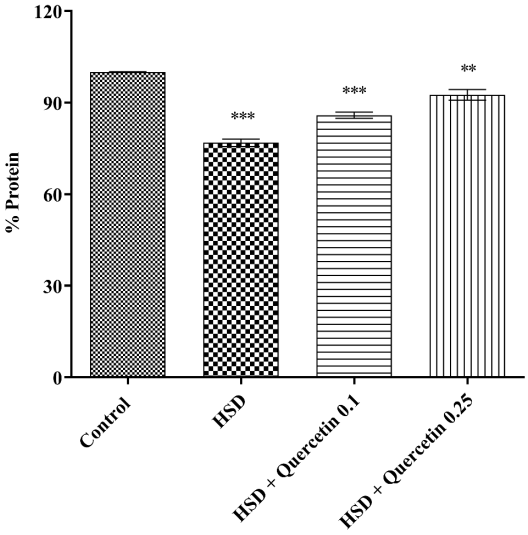

Figure S5a: Effects of Quercetin on HSD-fed Drosophila protein levels *p<0.05, **p<0.01, ***p<0.001 compared with control.

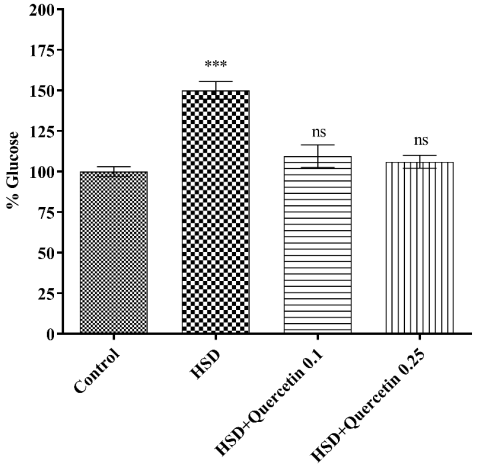

Figure S5b: Effects of Quercetin on HSD-fed Drosophila glucose levels *p<0.05, **p<0.01, ***p<0.001 compared with control.

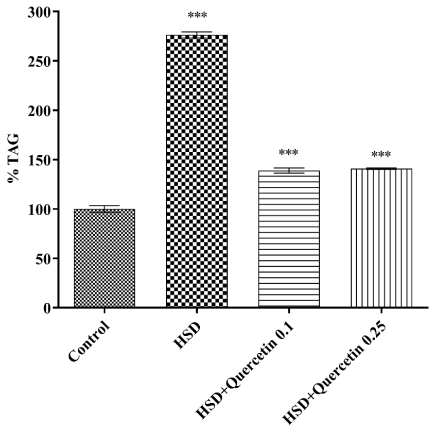

Figure S5c: Effects of Quercetin on HSD-fed Drosophila TAG levels.

Supplementary Table 1: Primers Used for Experiment.

Genes	Reverse sequence	Forward sequence	Reference
d-foxo	3'- TCGCCGAACTCAGTAACCAC-5'	3' – TCCTATCAAAGTAGAGGCGCA – 5'	(Westfall & Lomis, 2016)
p 38	3'- CAGGTGTCAAAGGCAGTTGTT-5'	3'-GCTCCCGGTACGTCCTCTT-5'	(Patel *et al.*, 2019)
Met	5'-GCCAGTAAGCATTACCAGCGAGAG-3'	5'-TGGAGGCAGTAGAACGAGGTGAC-3'	(Fu et al., 2022)
JHAMT	5'-GCCAGTAAGCATTACCAGCGAGAG-3'	5'-TGGAGGCAGTAGAACGAGGTGAC-3'	
ECR	5'-CGCTGGACTCGCACGACTATTG-3'	5'-CGCTCTGCTGCTGCTGACTTAG-3'	
Br	5'- GCAACAACAGCAGCAGCAACAG-3'	5'-GTGTGGTGGTGGGCGTATTGG-3'	
E74	5'- CGTCGGAGAGGAGAGTGGAGTG-3'	5'- TTGGTGTGCGTGTGCTGTGTAC-3'	
HMGR	5'- GTCCCATAAAGCCTCCACGC-3'	5'- TTGTGGAGTGGGCAGTGAGT-3'	
Kr-h1	5'- TGTGGCATGACCTTTGGCAG-3'	5'- CTCCAGAGGCGCCATTAAGC-3'	
β-actin	5'- TTGTCTGGGCAAGAGGATCAG-3'	5'-ACCACTCGCACTTGCACTTTC-3'	
Nrf2	5'-TTACATCTACGAGTACGCCGC-3',	5'-ACTGGAGCTCAAAACCGCTAA-3',	(Le & Inoue, 2021)
MPK2	5-GGCCACATAGCCTGTCATCT 3	5-ACCAGATACTCCGTGGCTTG 3	(Trindade de Paula *et al.*, 2016)

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