ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Aspirin-Mediated Reduction of Glucose Level and Inflammation in *Drosophila melanogaster*

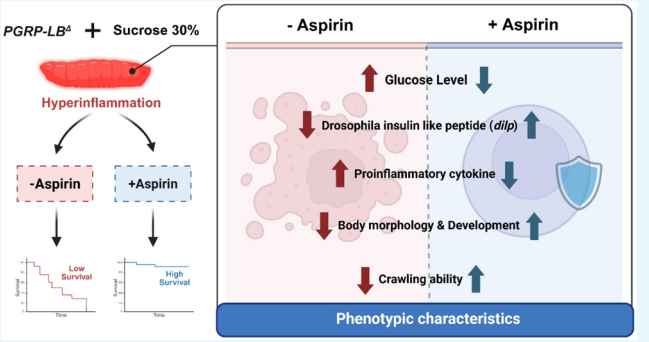
Muhammad Rayza Azmin, Habibie Habibie, Filmaharani Filmaharani, Alfreds Roosevelt, Anggun Nurhidayah, Muhammad Rasul Pratama, Widya Hardiyanti, Nadila Pratiwi Latada, Mukarram Mudjahid, Dewi Yuliana, and Firzan Nainu*

Cite This: *ACS Omega* 2025, 10, 18622−18628

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Diabetes mellitus (DM), particularly type 2 diabetes mellitus (T2DM), is a global health challenge marked by chronic hyperglycemia and inflammation, which contributes to both metabolic dysregulation and associated complications. Inflammation exacerbates T2DM by activating immune signaling pathways and promoting insulin resistance. This study aims to investigate the interplay between hyperglycemia and inflammation and to explore the therapeutic potential of aspirin in mitigating these processes using *Drosophila melanogaster* as a model organism. We utilized the *PGRP-LB*$^{\Delta}$ strain, which exhibits dysregulated immune responses due to the loss of the *PGRP-LB* gene, leading to a phenotype resembling human autoinflammatory conditions. Larvae of the *PGRP-LB*$^{\Delta}$ were fed a high-sucrose diet to induce increased glucose levels, mimicking the metabolic disturbances of T2DM. Aspirin, at different concentrations, was administered to assess its effects on high glucose level-induced inflammation. The results demonstrated that aspirin significantly improved hemolymph glucose levels, larval size, weight, and development. Additionally, aspirin enhanced larval mobility and reduced glucose level-associated immune dysfunction, as evidenced by changes in the expression of key immune and insulin-related genes. These findings highlight the utility of *D. melanogaster* as an effective and cost-efficient model to investigate the molecular mechanisms of T2DM and inflammation. The study also provides preliminary evidence for the potential of aspirin as an anti-inflammatory agent to modulate glucose levels and inflammation in T2DM, offering a promising avenue for therapeutic development.



1. INTRODUCTION

Diabetes mellitus (DM) is a major global health crisis, with the number of people affected by it steadily increasing. Currently, over 529 million people worldwide are living with diabetes, and this number is expected to rise significantly by 2050.[1] Type 2 diabetes mellitus (T2DM), the most common form, is frequently accompanied by severe microvascular and macrovascular complications, including retinopathy, nephropathy, cardiovascular disease, and neuropathy. These complications not only degrade the quality of life but also significantly increase the risk of mortality,[2] contributing to the growing burden on healthcare systems globally. Despite advancements in diabetes treatment, including the use of insulin analogues and newer drugs, the complexity of T2DM remains a significant challenge for effective management, particularly in addressing its underlying mechanisms and complications.

A key factor in the onset and progression of T2DM is uncontrolled hyperglycemia, which is characterized by persistently elevated blood glucose levels. Hyperglycemia is strongly associated with chronic inflammation, a hallmark of T2DM that exacerbates metabolic dysregulation and contributes to insulin resistance, one of the defining features of the disease.[3] The interplay between hyperglycemia and inflammation highlights the complex pathophysiology of T2DM and underscores the need for novel therapeutic strategies that target both metabolic and inflammatory pathways.

At the molecular level, hyperglycemia and oxidative stress activate the IκB kinase (IKK) pathway, leading to the activation of nuclear factor κB (NF-κB), a central regulator of inflammation. Additionally, cytokines can stimulate the signal transducer and activator of transcription 3 (STAT3) through the Jak1/2 pathway, further enhancing the inflammatory response.[4] These signaling pathways amplify the

Received: December 23, 2024
Revised: April 11, 2025
Accepted: April 22, 2025
Published: April 29, 2025

production of pro-inflammatory cytokines, contributing to a vicious cycle of inflammation that worsens the disease. Furthermore, hyperglycemia promotes the formation of advanced glycation end products (AGEs), which further exacerbate inflammation and insulin resistance, accelerating the progression of T2DM.[5,6]

Given the critical role of inflammation in T2DM, targeting inflammatory pathways represents a promising therapeutic approach. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, have shown potential in modulating these pathways. Aspirin works by inhibiting the *cyclooxygenase* (COX) enzyme, which plays a key role in the synthesis of pro-inflammatory prostaglandins. By suppressing prostaglandin production, aspirin reduces inflammation and its downstream effects.[7] Moreover, emerging evidence suggests that low-dose aspirin may improve insulin sensitivity, acting as an antihyperglycemic agent by mitigating inflammation in T2DM.[8,9] These findings highlight the potential of aspirin and other anti-inflammatory treatments to simultaneously address both reduced glucose levels and inflammation, offering a dual benefit for diabetes management.

In this study, we aim to provide proof of concept for the use of aspirin in mitigating glucose levels and inflammation in vivo using *Drosophila melanogaster* as a model system. The selection of *D. melanogaster* is based on its alignment with the 3R principles (Replacement, Reduction, Refinement),[10] as well as its numerous advantages as an invertebrate model.[11,12] *D. melanogaster* is an ideal organism for investigating complex diseases such as T2DM, as it is genetically tractable, has a short lifespan, and shares significant molecular pathways with humans.[13−15] Notably, *D. melanogaster* exhibits approximately 75% genetic similarity to humans, making it a powerful tool for translational research.[16]

In particular, we utilize the *PGRP-LB*$^{\Delta}$ of *D. melanogaster*, which carries a mutation in the *PGRP-LB* gene (peptidoglycan recognition protein-LB). *PGRP-LB* is a critical regulator of immune responses mediated by NF-κB, and its absence leads to dysregulated cytokine responses, mimicking the auto-inflammatory conditions observed in humans.[17−19] This model allows for the examination of the interplay between glucose levels and inflammation in a genetically accessible and simplified system. Additionally, this provides an opportunity to evaluate the therapeutic potential of aspirin in modulating these processes.

The primary objectives of this study are to evaluate the impact of increased glucose levels on inflammation in the *PGRP-LB*$^{\Delta}$ model and assess the effectiveness of aspirin in alleviating increased glucose level-induced inflammation. We hypothesize that aspirin will improve glucose metabolism and reduce inflammation in *PGRP-LB*$^{\Delta}$ larvae exposed to a high-sucrose diet and that these effects will be mediated through the modulation of immune and insulin-related signaling pathways. By using *D. melanogaster* as a model, this study aims to provide valuable insights into the molecular mechanisms of high glucose levels and inflammation as well as contribute to the development of novel therapeutic strategies for T2DM.

2. MATERIALS AND METHODS

2.1. *Drosophila* Stocks. In this study, fruit fly *D. melanogaster PGRP-LB*$^{\Delta}$, obtained from the Host Defense and Responses Laboratory at Kanazawa University, Japan, was used as the model organism. The flies were maintained on a standard diet under controlled conditions, including 60% relative humidity, a constant temperature of 25 °C, and a 12:12-h light−dark cycle to support optimal growth and development.

2.2. Materials. Sucrose (CAS No: 57−50−1, Smart Lab, Indonesia) and aspirin (CAS No. 50−78−2, Sigma-Aldrich) were used as the primary components for preparing the high-sugar diet for *D. melanogaster*.

2.3. Preparation of *Drosophila* Food. The *Drosophila* diet was categorized into five groups, with the composition of each group outlined in Table 1. A high-sugar diet (HSD) was formulated to induce high glucose levels in flies, and aspirin was included as a treatment. Aspirin was dissolved in 70% ethanol and incorporated into the food to achieve final concentrations of 0.05, 0.5, and 5 μM.[20] The complete experimental design is illustrated in Figure 1.

Table 1. Food Composition and Treatment

ingredients	normal diet (ND)	high-sugar diet (HSD)	high-sugar diet (HSD) + treatment
corn meal (g)	7.5	7.5	7.5
yeast (g)	2.5	2.5	2.5
agar (g)	0.9	0.9	0.9
sucrose (g)	4.5	30	30
propionic acid (μL)	400	400	400
methyl paraben (μL)	450	450	450
EtOH (%)			3.5
aspirin (μM)			0.05 μM
			0.5 μM
			5 μM
water (mL)	100	100	100

2.4. Measurement of *hemolymph* Glucose Levels in *D. melanogaster*. Approximately 70 third-instar larvae were collected to extract hemolymph for glucose level measurement. The larvae were placed in microtubes and homogenized by using a micropestle. The resulting hemolymph was centrifuged at 13,000 rpm for 2 min to separate the supernatant. Subsequently, 10 μL of the hemolymph was pipetted into a microcentrifuge tube containing 1000 μL of the glucose oxidase−peroxidase aminoantipyrine (GOD-PAP) reagent (Glory Diagnostics, Barcelona, Spain). The mixture was thoroughly homogenized and incubated at 37 °C for 10 min. Absorbance was then measured using a spectrophotometer (Shimadzu UV−vis 1800, Kyoto, Japan) at a wavelength of 500 nm. A standard glucose solution with a concentration of 100 mg/dL was used as a reference to calculate absorbance.[21]

2.5. Measurement of Larval Length, Width, and Body Weight. Body weight measurements were conducted by weighing ten third-instar *PGRP-LB*$^{\Delta}$ larvae from each experimental group using an analytical balance. The average body weight for each group was then calculated. Larval length and width were subsequently measured by using an analytical caliper and a microscope. The experimental groups included flies fed a normal diet, a high-sugar diet (HSD), HSD with solvent control, HSD with aspirin at 0.05 μM, HSD with aspirin at 0.5 μM, and HSD with aspirin at 5 μM.[21]

2.6. Larval Crawling Assay. The crawling assay was conducted using third-instar *PGRP-LB*$^{\Delta}$ larvae across all treatment groups with three larvae per group. Each larva was

https://doi.org/10.1021/acsomega.4c11509
ACS Omega 2025, 10, 18622−18628

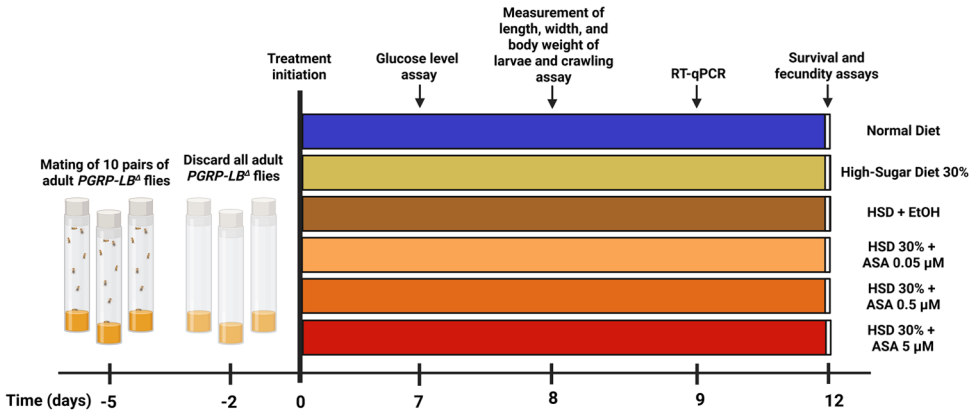


Figure 1. Experimental design. Six groups of 3rd instar larvae were given fly food containing a normal diet, HSD, HSD + EtOH, or HSD + ASA (0.05 μM, 0.5 μM, and 5 μM). ASA, acetylsalicylic acid; EtOH, ethanol; HSD, high-sugar diet.

Table 2. Primers Used in the RT-qPCR Assay

genes	forward primer (5′-3′)	reverse primer (5′-3′)
dilp3	GCTGCGCAATCGCTTCTACT	TGGTGGAGTGGGCTTCATG
dilp6	ATCCTTATGATCGGCGGTGT	GTTCACGGGGTCCAAAGTTC
drs	CGTGAGAACCTT TTCCAATATGATG	TCCCAGGACCACCAGCAT
attaA	CCCGGAGTGAAGGATG	GTTGCTGTGCGTCAAG
rp49	GACGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG

placed on a glass Petri dish containing an agar medium overlaid with graph paper. Larval movement was recorded by measuring the distance traveled in millimeters per minute, determined by counting the number of grid squares crossed within 1 min.[22]

2.7. Survival Assay. The survival assay was designed to assess the developmental progression from larvae to adult flies. Third-instar *PGRP-LB*$^{\Delta}$ larvae reared on HSD were transferred into vials containing one of the following diets: normal diet, HSD (negative control), HSD supplemented with solvent (solvent control), or HSD supplemented with aspirin treatments. Each vial contained ten larvae. The experiment was conducted at 25 °C with the diet replaced every 3 days. Daily observations were made to document the number of larvae that successfully metamorphosed into pupa and the number of pupae that emerged as adult flies. Mortality was recorded daily for each experimental group until all flies either developed into adults or died.[23]

2.8. Fecundity Assay. For the fecundity assay, a group of five virgin female and five male *PGRP-LB*$^{\Delta}$ flies were placed in a treatment vial. The experiment consisted of three groups, with the flies allowed 5 days for reproduction to assess fecundity in each treatment group. After this period, all parental flies were removed. The number of pupae and adult flies that emerged from each group was counted for analysis. Virgin female flies, defined as females that had never mated and were 5−7 h posteclosion from the pupal stage, were used for this experiment.[24]

2.9. Gene Expression Analysis. Ten *PGRP-LB*$^{\Delta}$ larvae, previously subjected to treatment, were placed in Treff tubes for total RNA isolation using the PureLink RNA Mini Kit (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad), following the manufacturer's instructions. Gene expression levels were quantified using quantitative reverse transcription PCR (RT-qPCR) with the Universal One-Step RT-qPCR Kit (Luna, New England Biolabs, Inc., MA), in accordance with the manufacturer's protocol. The RT-qPCR reactions were performed in a 20 μL reaction volume, and the RT-qPCR analysis for target genes (primer sequences are listed in Table 2) was conducted in a 10 μL reaction volume using a RotorGene Q (Qiagen, Germany). The procedure involved one cycle at 50 °C for 10 min, followed by 95 °C for 2 min, and then 40 cycles at 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 30 s. A standard melt curve analysis was performed to ensure the specific amplification of the expected product. The RNA levels of host ribosomal protein *rp49*, used as an internal control, were also measured using *rp49* primers, following the same protocol applied to the target genes.

2.10. Data Analysis. All data obtained from both phenotypic and molecular tests were analyzed using Prism 9. (GraphPad Software, Boston). The data were presented in the form of bar graphs and statistically analyzed using one-way ANOVA, followed by Tukey's posthoc test. The results are expressed as mean ± standard deviation (mean ± SD), with statistical significance determined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Reduction of Glucose Level in *PGRP-LB*$^{\Delta}$ Flies by Aspirin Is Accompanied by the Downregulation of Pro-Inflammatory Peptides. Although the interaction between inflammation and hyperglycemia has been widely reported, the extent of the host immune system's role in regulating metabolic homeostasis remains unclear. To address this, we utilized *D. melanogaster* mutants lacking *PGRP-LB*, a key negative regulator of the Imd pathway that plays a crucial role in modulating inflammation. Within the Imd immune pathway, *PGRP-LB* and *PGRP-SC* act as essential negative regulators by degrading bacterial peptidoglycan, thereby preventing excessive immune activation.[25] Unlike *PGRP-SC*, which acts locally in epithelial tissues,[26] *PGRP-LB* functions systemically in the gut and fat body,[19] with its loss leading to a stronger inflammatory response.[25] The absence of this protein in the *PGRP-LB*$^{\Delta}$ causes excessive cytokine activation, which

https://doi.org/10.1021/acsomega.4c11509
ACS Omega 2025, 10, 18622−18628

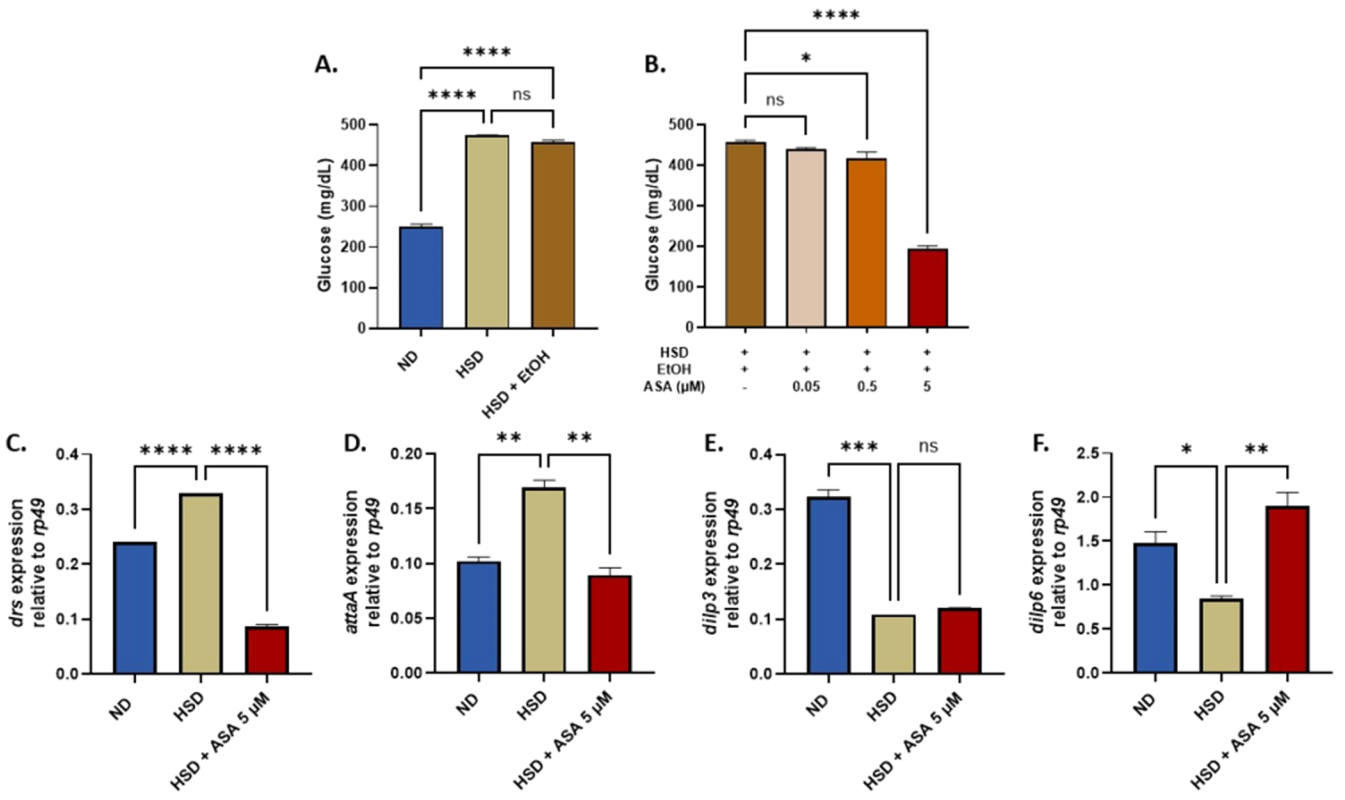


Figure 2. Increased glucose level following aspirin treatment. The glucose level in the larval body increased due to HSD induction (A), while aspirin treatment effectively reduced the glucose level in a dose-dependent manner (B). The reduction in the glucose level is associated with the regulation of gene expression of *drs* (C), *attaA* (D), *dilp3* (E), and *dilp6* (F). ND, normal diet; HSD, high-sugar diet; EtOH, ethanol; ASA, aspirin; ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ****, and $p < 0.0001$ (means ± SD, $n = 3$).

negatively impacts survival.[27] To investigate the effects of high glucose levels on immune activity in this mutant, we administered a high-sugar diet to the *PGRP-LB*$^{\Delta}$ flies.

In this study, the *PGRP-LB*$^{\Delta}$ was exposed to a 30% sucrose diet to induce high glucose-level conditions. The results showed a significant increase in glucose levels in the hemolymph of larvae fed a high-sugar diet (HSD) compared to that of the untreated control (Figure 2A). This finding confirms that the 30% sucrose treatment effectively increased the glucose level in *PGRP-LB*$^{\Delta}$. These results are consistent with previous research showing that the use of 30% sucrose can increase *hemolymph* glucose levels.[28] Meanwhile, the addition of ethanol as a solvent did not affect glucose levels, indicating that the solvent is inert. On the other hand, it is important to note that the aspirin treatment in this condition successfully reduced the glucose level in the *PGRP-LB* mutant (Figure 2B). To further investigate the mechanism underlying this effect, we analyzed the molecular expression of immunity- and metabolism-related genes in the high glucose-level *PGRP-LB*$^{\Delta}$ model during aspirin treatment.

Molecular analysis indicates that aspirin's antihyperglycemic effects may be closely linked to its ability to suppress inflammation. This is supported by RT-qPCR data showing the expression of *drs* (Figure 2C) and *attaA* (Figure 2D) genes in the *PGRP-LB*$^{\Delta}$. Drosomycin and Attacin A are cytokine-like peptides in *D. melanogaster*, regulated by the NF-κB Toll and Imd signaling pathways.[29] High-sugar diets are known to dysregulate inflammatory gene expression in *D. melanogaster*.[30] Under high-sugar diet conditions, aspirin administration significantly reduced the expression of these inflammatory markers, highlighting its potential to mitigate inflammation-associated metabolic dysfunction.

Furthermore, an analysis was conducted on metabolic genes such as *dilp3* and *dilp6*. Both of these genes are part of the insulin-like peptides (*dilps*) found in *D. melanogaster*. The *dilp3* gene functions similarly to insulin and insulin-like growth factors (IGF) in mammals, playing an important role in regulating energy metabolism, maintaining glucose balance, supporting growth processes, and helping the body adapt to stress.[31] Meanwhile, *dilp6* also plays a role in regulating metabolism, growth, and the body's response to environmental changes, such as nutrient availability, including glucose, which is the main energy source in metabolism.[32,33] A high-sugar diet is known to trigger insulin resistance.[34] Meanwhile, aspirin was observed to increase *dilp6* but not affect *dilp3* (Figure 2E,F), indicating the recovery of metabolic function regulated by aspirin through pathways involved in insulin secretion in *PGRP-LB*$^{\Delta}$.

3.2. Aspirin-Induced Restoration of Body Size, Weight, and Crawling Activity of High Glucose-Treated *Drosophila* Larvae.

The increase in glucose levels in the body's circulation has been known to trigger metabolic disorders. It has been previously reported that in conditions of hyperglycemia, where the body cannot effectively use glucose as an energy source, fat reserves and muscle protein are used as alternative energy sources, leading to weight loss.[35,36] Additionally, hyperglycemia also affects motor function, as reported by Nayak and Mishra, which shows that oxidative stress due to hyperglycemia damages nerve cells and inhibits signal transmission.[22] Therefore, further testing was conducted

https://doi.org/10.1021/acsomega.4c11509
ACS Omega 2025, 10, 18622−18628

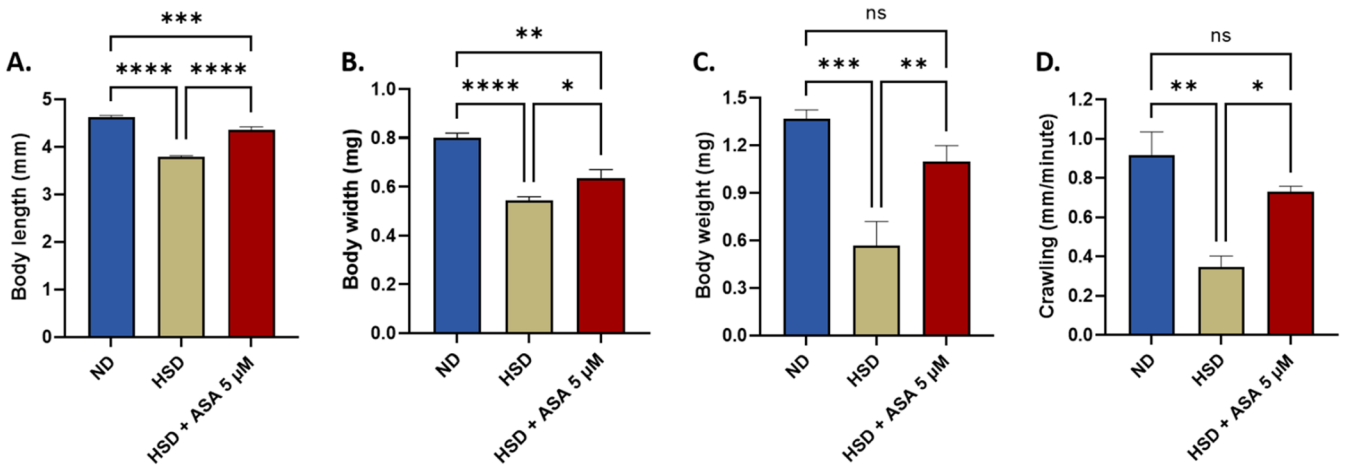


Figure 3. Improvement in body size, body weight, and crawling activity after aspirin administration. HSD exposure is capable of reducing body size, weight, and crawling activity. After aspirin administration, it effectively increases body length (A), body width (B), body weight (C), and crawling activity (D). ND, normal diet; HSD, high-sugar diet; ASA, aspirin; ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, and $p < 0.0001$ (means ± SD, $n = 3$).

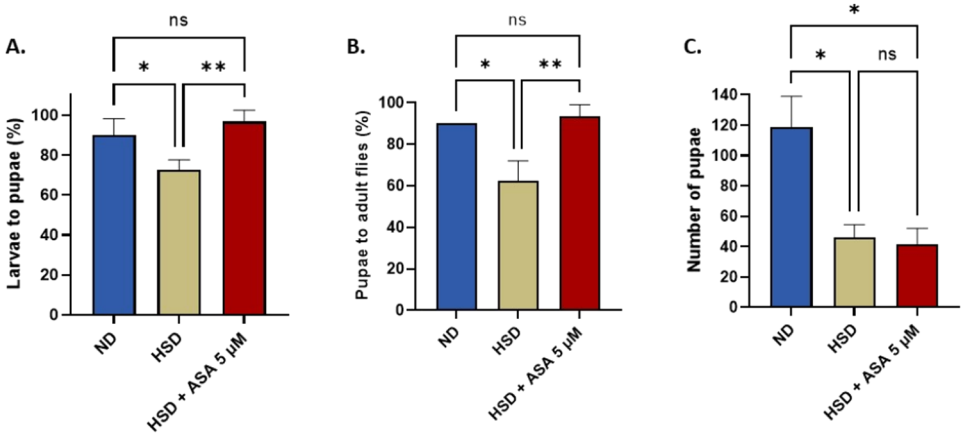


Figure 4. Improvement in survival after aspirin administration but not in fecundity. HSD exposure was able to reduce the survival from larva to adult fly and fecundity. After aspirin administration, it effectively improved the survival from larva to pupa (A) and improved the survival from pupa to adult fly (B) but not in fecundity (C). ND, normal diet; HSD, high-sugar diet; ASA, aspirin; ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$ (means ± SD, $n = 3$).

to examine body weight and crawling activity in *PGRP-LB*$^{\Delta}$ treated with high glucose food both before and after treatment with aspirin.

Hyperglycemia is known to affect body size in both humans and model animals, such as mice and *D. melanogaster*.[37,38] In this study, the effect of increased glucose level on body length and width was also tested on *PGRP-LB*$^{\Delta}$ *D. melanogaster* larvae exposed to a high-sugar diet. Body length measurements showed a significant decrease after the high-sugar diet treatment (Figure 3A). A similar finding was observed in the body width of the larvae, which decreased due to the increased glucose level induced by the high-sugar diet (Figure 3B). However, the administration of aspirin was able to significantly increase the body length (Figure 3A) and body width (Figure 3B) of the larvae.

PGRP-LB$^{\Delta}$ larvae were subjected to a high-sugar diet to induce a condition of an increased glucose level. The results show that this treatment caused a decrease in the larvae's body weight (Figure 3C). This is consistent with the findings of Meshrif et al., who reported that a high-sugar diet in larvae can trigger a decrease in body weight.[37] However, the administration of aspirin significantly increased the body weight of the *PGRP-LB*$^{\Delta}$ larvae (Figure 3C), demonstrating its potential in reducing the negative effects of an increased glucose level. In addition to its effect on body weight, a high-sugar diet treatment also induces a decrease in crawling activity in *PGRP-LB*$^{\Delta}$ larvae (Figure 3D). The disturbed crawling activity indicates motor function impairment, which is likely caused by oxidative stress on nerve cells. This stress hampers nerve signal transmission, resulting in irregular movement patterns in the larvae.[22] However, the administration of aspirin can enhance the crawling activity of *PGRP-LB* mutant larvae (Figure 3D).

3.3. Aspirin Improves the Development but Not the Fecundity of *PGRP-LB*$^{\Delta}$ during HSD Consumption. High glucose levels not only affect the size and weight of larvae but also contribute to developmental delays. This is consistent with the research by Meshrif et al., which reported that larvae on a high-sugar diet showed developmental delays up to the pupal stage. This influence indicates that a high-sugar diet can slow down the overall developmental process from larvae to adult flies.[37] The development test of *PGRP-LB*$^{\Delta}$ from larvae to pupae showed a significant decrease due to high-sugar diet

https://doi.org/10.1021/acsomega.4c11509
ACS Omega 2025, 10, 18622−18628

treatment (Figure 4A). However, the administration of aspirin significantly improved the development of larvae to pupae (Figure 4A). A similar finding was observed in the developmental stage from pupae to adult flies, where an increased glucose level caused a decrease in development (Figure 4B), which could then be mitigated by aspirin (Figure 4B).

The increase in glucose level induced by high-sugar diet (HSD) treatment in virgin female *PGRP-LB*$^{\Delta}$ flies causes a significant decrease in reproductive rates (Figure 4C). It has been reported that a high-sugar diet, particularly one containing sucrose, significantly reduces reproduction in *D. melanogaster*. This comparison confirms that a high-sugar content in the diet consistently adversely affects reproductive ability.[39] Flies experiencing an increase in glucose level were treated with aspirin. The results show that aspirin does not affect reproduction (Figure 4C). However, unlike the positive effects of aspirin on other parameters, such as body weight, body morphology, *crawling* activity, and development, the administration of aspirin does not show a significant effect in increasing the reproduction of virgin female *PGRP-LB*$^{\Delta}$ flies.

4. CONCLUSIONS

This study demonstrates that a high-sugar diet in *PGRP-LB*$^{\Delta}$ *D. melanogaster* induces inflammation and significantly affects physiological homeostasis, as evidenced by increased body glucose levels, reduced insulin production, impaired larval growth, and decreased fertility. However, aspirin administration effectively lowered glucose levels and improved growth and motor activity, although it did not influence fertility. Additionally, aspirin modulated the expression of inflammation- and metabolism-related genes, highlighting its potential in mitigating metabolic disorders associated with hyperglycemia.

AUTHOR INFORMATION

Corresponding Author

Firzan Nainu − *Department of Pharmacy, Faculty of Pharmacy and Unhas Fly Research Group, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*; orcid.org/0000-0003-0989-4023; Email: firzannainu@unhas.ac.id

Authors

Muhammad Rayza Azmin − *Postgraduate Program in Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Habibie Habibie − *Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Filmaharani Filmaharani − *Postgraduate Program in Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Alfreds Roosevelt − *Postgraduate Program in Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Anggun Nurhidayah − *Postgraduate Program in Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Muhammad Rasul Pratama − *Unhas Fly Research Group, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Widya Hardiyanti − *Unhas Fly Research Group, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Nadila Pratiwi Latada − *Unhas Fly Research Group, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Mukarram Mudjahid − *Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

Dewi Yuliana − *Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar 90231, Indonesia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c11509

Author Contributions

M.R.A. and F.N. were responsible for conceptualization; M.R.A., H.H., and F.N. were responsible for methodology; M.R.A., F.F., A.R., A.N., M.R.P., W.H., N.P.L., M.M., D.Y., and F.N. were responsible for data curation and formal analysis; M.R.A., N.P.L., and F.N. were responsible for writing the original draft preparation; M.R.A., H.H., N.P.L., M.M., and F.N. were responsible for writing, review, and editing; M.R.A. was responsible for visualization; and H.H. and F.N. were responsible for supervision. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to Prof. Takayuki Kuraishi from Kanazawa University, Japan, for kindly providing the *D. melanogaster* used in this study. The authors also extend their appreciation to Prof. Elly Wahyudin from the Faculty of Pharmacy, Hasanuddin University, Indonesia, for her invaluable support in facilitating our research activities at the Biofarmaka Laboratory. Additionally, the authors would like to thank Jumriani and Ratnawati for their insightful discussions throughout the course of the experiments.

REFERENCES

(1) Ong, K. L.; Stafford, L. K.; McLaughlin, S. A.; Boyko, E. J.; Vollset, S. E.; Smith, A. E.; Dalton, B. E.; Duprey, J.; Cruz, J. A.; Hagins, H.; Lindstedt, P. A.; Aali, A.; Abate, Y. H.; Abate, M. D.; Abbasian, M.; Abbasi-Kangevari, Z.; AbbasiKangevari, M.; ElHafeez, S. A.; Abd-Rabu, R.; Vos, T.; et al. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet* **2023**, *402* (10397), 203−234.

(2) Forbes, J. M.; Cooper, M. E. Mechanisms of diabetic complications. *Physiol. Rev.* **2013**, *93* (1), 137−188.

(3) International Diabetic Federation. *International Diabetic Federation Diabetic Atlas*, IDF2021.

(4) Evans, J. L.; Goldfine, I. D.; Maddux, B. A.; Grodsky, G. M. Oxidative stress and stress activated signaling pathways a unifying hypothesis of type 2 diabetes. *Endocr. Rev.* **2002**, *23* (5), 599−622.

(5) Byun, K.; Yoo, Y.; Son, M.; Lee, J.; Jeong, G. B.; Park, Y. M.; Salekdeh, G. H.; Lee, B. Advanced glycation end products produced systemically and by macrophages A common contributor to inflammation and degenerative diseases. *Pharmacol. Ther.* **2017**, *177*, 44−55.

(6) Goldberg, R. B. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in

development of diabetes and its complications. *J. Clin. Endocrinol. Metab.* **2009**, *94* (9), 3171−3182.
(7) Hammadi, S. H.; Al-Ghamdi, S. S.; Yassien, A. I.; Al-Hassani, S. D. Aspirin and Blood Glucose and Insulin Resistance. *Open J. Endocr. Metab. Dis.* **2012**, *02* (02), 16−26.
(8) Abdelsadik, A. P.; Amin, M. M. Low-dose aspirin improves glucose uptake and attenuates inflammation in rats fed high-fat diet. *Egypt. Pharm. J.* **2018**, *17* (3), 171−179.
(9) Hundal, R. S.; Petersen, K. F.; Mayerson, A. B.; Randhawa, P. S.; Inzucchi, S.; Shoelson, S. E.; Shulman, G. I. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J. Clin. Invest.* **2002**, *109* (10), 1321−1326.
(10) Russell, W. M. S.; Burch, R. L. *The Principles of Humane Experimental Technique.*; Methuen: London, 1959.
(11) Munkácsy, E.; Pickering, A. M. Model Organisms (Invertebrates). In *Handbook of the Biology of Aging*; Academic Press, 2021; pp 199−217.
(12) Correa, C. L.; de Andrade Gomes, C. A. B.; Mello, A. D. A.; Nogueira, N. D. S.; Medeiros, T. B.; de Barros, C. M.; Allodi, S. Use of invertebrates to model chemically induced parkinsonism-symptoms. *Biochem. Soc. Trans.* **2023**, *51* (1), 435−445.
(13) Pandey, U. B.; Nichols, C. D. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. *Pharmacol Rev.* **2011**, *63*, 411−436.
(14) Verheyen, E. M. The power of Drosophila in modeling human disease mechanisms. *Dis. Models Mech.* **2022**, *15* (3), No. dmm049549.
(15) Chen, D.; Yang, J.; Xiao, Z.; Zhou, S.; Wang, L. A diet-induced type 2 diabetes model in Drosophila. *Sci. China Life Sci.* **2021**, *64*, 326−329.
(16) Vidal, M.; Arch, M.; Fuentes, E.; Cardona, P. J. Drosophila melanogaster experimental model to test new antimicrobials: a methodological approach. *Front. Microbiol.* **2024**, *15*, No. 1478263.
(17) Buchon, N.; Silverman, N.; Cherry, S. Immunity in Drosophila melanogaster from Microbial Recognition to Whole Organism Physiology. *Nat. Rev. Immunol.* **2014**, *14* (12), 796−810.
(18) Orlans, J.; Vincent-Monegat, C.; Rahioui, I.; Sivignon, C.; Butryn, A.; Soulère, L.; Da Silva, P.; et al. PGRP-LB: An inside view into the mechanism of the amidase reaction. *Int. J. Mol. Sci.* **2021**, *22* (9), No. 4957.
(19) Zaidman-Rémy, A.; Hervé, M.; Poidevin, M.; Pili-Floury, S.; Kim, M. S.; Blanot, D.; Lemaitre, B.; et al. The Drosophila amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity* **2006**, *24* (4), 463−473.
(20) Nainu, F.; Bahar, M. A.; Sartini, S.; Rosa, R. A.; Rahmah, N.; Kamri, R. A.; Rumata, N. R.; Yulianty, R.; Wahyudin, E. Proof-of-Concept Preclinical Use of Drosophila melanogaster in the Initial Screening of Immunomodulators. *Sci. Pharm.* **2022**, *90* (1), No. 11.
(21) Lourido, F.; Quenti, D.; Salgado-Canales, D.; Tobar, N. Domeless receptor loss in fat body tissue reverts insulin resistance induced by a high-sugar diet in Drosophila melanogaster. *Sci. Rep.* **2021**, *11*, No. 3263.
(22) Nayak, N.; Mishra, M. High fat diet induced abnormalities in metabolism, growth, behavior, and circadian clock in Drosophila melanogaster. *Life Sci.* **2021**, *281*, No. 119758.
(23) Ismail, J. N.; Mantash, S.; Hallal, M.; Jabado, N.; Khoueiry, P.; Shirinian, M. Phenotypic and transcriptomic impact of expressing mammalian TET2 in the Drosophila melanogaster model. *Epigenetics* **2023**, *18*, No. 2192375.
(24) Koliada, A.; Gavrilyuk, K.; Burdylyuk, N.; Strilbytska, O.; Storey, K. B.; Kuharskii, V.; et al. Mating status affects Drosophila lifespan, metabolism and antioxidant system. *Comp. Biochem. Physiol., Part A:Mol. Integr. Physiol.* **2020**, *246*, No. 110716.
(25) Paredes, J. C.; Welchman, D. P.; Poidevin, M.; Lemaitre, B. Negative regulation by amidase PGRPs shapes the Drosophila antibacterial response and protects the fly from innocuous infection. *Immunity* **2011**, *35* (5), 770−779.
(26) Werner, T.; Liu, G.; Kang, D.; Ekengren, S.; Steiner, H.; Hultmark, D. A family of peptidoglycan recognition proteins in the fruit fly Drosophila melanogaster. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97* (25), 13772−13777.
(27) Kurata, S. Recognition of infectious non-self and activation of immune responses by peptidoglycan recognition protein (PGRP)-family members in Drosophila. *Dev. Comp. Immunol.* **2004**, *28* (2), 89−95.
(28) Asiimwe, O. H.; Rubaihayo, J.; Sulaiman, S. O.; Osuwat, L. O.; Kasozi, K. I. A protein restricted diet induces a stable increased fat storage phenotype in flies. *Toxicol. Rep.* **2023**, *10*, 706−713.
(29) Lemaitre, B.; Hoffmann, J. The host defense of Drosophila melanogaster. *Annu. Rev. Immunol.* **2007**, *25*, 697−743.
(30) Yu, S.; Zhang, G.; Jin, L. H. A high-sugar diet affects cellular and humoral immune responses in Drosophila. *Exp. Cell Res.* **2018**, *368* (2), 215−224.
(31) Nässel, D. R.; Broeck, J. V. Insulin/IGF signaling in Drosophila and other insects: factors that regulate production, release, and post-release action of the insulin-like peptides. *Cell. Mol. Life Sci.* **2016**, *73* (15), 271−290.
(32) Okamoto, N.; Yamanaka, N.; Yagi, Y.; Nishida, Y.; Kataoka, H.; O'Connor, M. B.; Mizoguchi, A. A fat body-derived IGF-like peptide regulates postfeeding growth in Drosophila. *Dev. Cell* **2009**, *17* (6), 885−891.
(33) Nwangwu, S.; Joel, J. S.; Kingsley, O.; Okwuonu, U.; Sunday, A.; Chinenye, O. Effects of Anticoagulants on Fasting Blood Glucose of Diabetics and Non-Diabetics Individuals, as well as Random Blood Glucose of Apparently Healthy Individuals, Determined by One Touch Ultra Glucometer. *Int. J. Pharm. Clin. Sci.* **2012**, *2*, 11−13.
(34) Pasco, M. Y.; Léopold, P. High sugar-induced insulin resistance in Drosophila relies on the lipocalin Neural Lazarillo. *PLoS One* **2012**, *7* (5), No. e36583.
(35) Holt, R. I.; DeVries, J. H.; Hess-Fischl, A.; Hirsch, I. B.; Kirkman, M. S.; Klupa, T.; Peters, A. L.; et al. The management of type 1 diabetes in adults. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* **2021**, *44* (11), 2589−2625.
(36) Renaud, J.; Bassareo, V.; Beaulieu, J.; Pinna, A.; Schlich, M.; Lavoie, C.; Martinoli, M. G.; et al. Dopaminergic neurodegeneration in a rat model of long-term hyperglycemia: preferential degeneration of the nigrostriatal motor pathway. *Neurobiol. Aging* **2018**, *69*, 117−128.
(37) Meshrif, W. S.; El Husseiny, I. M.; Elbrense, H. Drosophila melanogaster as a low-cost and valuable model for studying type 2 diabetes. *J. Exp. Zool., Part A* **2022**, *337* (5), 457−466.
(38) Géminard, C.; Arquier, N.; Layalle, S.; Bourouis, M.; Slaidina, M.; Delanoue, R.; Léopold, P.; et al. Control of metabolism and growth through insulin-like peptides in Drosophila. *Diabetes* **2006**, *55*, S5−S8.
(39) Lushchak, O. V.; Gospodaryov, D. V.; Rovenko, B. M.; Yurkevych, I. S.; Perkhulyn, N. V.; Lushchak, V. I. Specific dietary carbohydrates differentially influence the life span and fecundity of Drosophila melanogaster. *J. Gerontol., Ser. A* **2014**, *69* (1), 3−12.

https://doi.org/10.1021/acsomega.4c11509
ACS Omega 2025, 10, 18622−18628