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Original Article

Anti-aging and immunomodulatory role of caffeine in *Drosophila* larvae

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Abstract

Drug repurposing is a promising approach to identify new pharmacological indications for drugs that have already been established. However, there is still a limitation in the availability of a high-throughput *in vivo* preclinical system that is suitable for screening and investigating new pharmacological indications. The aim of this study was to introduce the application of *Drosophila* larvae as an *in vivo* platform to screen drug candidates with anti-aging and immunomodulatory activities. To determine whether *Drosophila* larvae can be utilized for assessing anti-aging and immunomodulatory activities, phenotypical and molecular assays were conducted using wildtype and mutant lines of *Drosophila*. The utilization of mutant lines (*PGRP-LB*$^{\Delta}$ and *Psh[1];;ModSP[KO]*) mimics the autoinflammatory and immunodeficient conditions in humans, thereby enabling a thorough investigation of the effects of various compounds. The phenotypical assay was carried out using survival and locomotor observation in *Drosophila* larvae and adult flies. Meanwhile, the molecular assay was conducted using the RT-qPCR method. *In vivo* survival analysis revealed that caffeine was relatively safe for *Drosophila* larvae and exhibited the ability to extend *Drosophila* lifespan compared to the untreated controls, suggesting its anti-aging properties. Further analysis using the RT-qPCR method demonstrated that caffeine treatment induced transcriptional changes in the *Drosophila* larvae, particularly in the downstream of NF-κB and JAK-STAT pathways, two distinct immune-related pathways homologue to humans. In addition, caffeine enhanced the survival of *Drosophila* autoinflammatory model, further implying its immunosuppressive activity. Nevertheless, this compound had minimal to no effect on the survival of *Staphylococcus aureus*-infected wildtype and immunodeficient *Drosophila*, refuting its antibacterial and immunostimulant activities. Overall, our results suggest that the anti-aging and immunosuppressive activities of caffeine observed in *Drosophila* larvae align with those reported in mammalian model systems, emphasizing the suitability of *Drosophila* larvae as a model organism in drug repurposing endeavors, particularly for the screening of newly discovered chemical entities to assess their immunomodulatory activities before proceedings to investigations in mammalian animal models.

Keywords: Anti-aging, caffeine, drug repurposing, *in vivo*, immunomodulator

Introduction

In recent years, obtaining drug candidates with novel mechanisms of action has been an exciting yet challenging process [1]. Despite successful preclinical assessments, up to 90% of drug

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candidates failed to deliver promising results in clinical trials [2], jeopardizing the pipeline of drug discovery and development. Strikingly, some newly discovered chemical entities have failed to pass phase 1 clinical trials [3], suggesting that these compounds might not be properly safe for humans and, to some extent, implying that animal toxicity studies may not deliver reliable results. To deal with this, various aspects have been evaluated to find the critical components of drug discovery that are subject to modification [2,3]. One of the solutions is the emergence of a drug repurposing initiative, taking advantage of the already available medicinal compounds/drugs that have passed at least phase 1 clinical trials [4,5]. Using this approach, one may swiftly discover new pharmacological indications without having to spend much resources [4]. To achieve the purpose of drug repurposing, it is critical to develop a high-throughput *in vivo* preclinical screening system suitable for the investigation of new pharmacological indications.

Fruit fly *Drosophila melanogaster* has been introduced as an emerging model in drug discovery and repurposing. With its genetic similarity to humans, this insect can support drug discovery efforts with fewer ethical considerations and reasonable experimental costs [6-9]. We have previously shown that adult *Drosophila* can be used for this purpose [10,11]. However, special equipment such as CO_2 system shall always be used to anesthetize adult *Drosophila* prior to use in the experiments. By using *Drosophila* larvae, the utilization of such equipment can be avoided, thus potentially reducing the cost of the preclinical assays.

Caffeine has a structure similar to adenine, which functions as an adenosine receptor antagonist that delays fatigue and drowsiness [12]. In addition, caffeine has been reported to alleviate the oxidative stress condition and prevent the cross-linking between DNA and reactive oxygen species (ROS) [13] as well as inhibit the photoaging-related enzymes [14], which might contribute to its anti-aging properties. Many studies have documented the pharmacological activities of caffeine as an adenosine receptor ligand, such as in the alleviation of anxiety effects and improvement of locomotor activity, memory, circadian rhythms, and neuroprotective effects [15-18]. Most importantly, caffeine has been considered as a promising immunomodulator due to its documented effect on the modulation of the immune system [19,20]. Exploration of the anti-aging and immunopharmacological effects of this widely known compound with good safety profiles shall provide important insights into the potential of our novel *in vivo Drosophila* larvae platform in drug repurposing. The aim of this study was to demonstrate the potential of using *D. melanogaster* larvae as an *in vivo* screening system for assessing anti-aging and immunomodulatory compounds. For this proof-of-principle study, caffeine was used. Hence, future application of screening and investigation of the immunomodulatory effects of novel drug candidates using this newly established platform shall offer beneficial pharmaceutical prospects.

Methods

Fly stocks and chemicals used in the study

D. melanogaster wildtype (Oregon R) and mutant (*PGRP-LB*$^{\Delta}$, and *Psh[1];;ModSP[KO])* lines were obtained from the Laboratory Host Defense and Responses (Kanazawa University, Japan). Flies were maintained in cornmeal-agar food (standard) and stored at 25°C for more than 20 inbred generations prior to use in the study. The caffeine used in the study was of pharmaceutical grade. Caffeine (Soho Nootropics, Logistics LLC, Arizona, U.S.) was prepared to obtain concentrations of 0.16, 0.8, and 4 mM in the fly food.

Toxicity assay

Toxicity assay was carried out based on the previously reported procedure [21], with slight modifications. Briefly, 200 of second instar larvae were selected and transferred into fly food (ten larvae per group and five replications for each group). The larvae were further divided into four groups: untreated control and caffeine-treated (0.16, 0.8, and 4 mM), maintained at 25°C and observed daily. The number of larvae that succeeded in developing into pupae and the number of adult flies that were enclosed from pupae were counted.

Preparation of bacterial strain and establishment of infection model using *Drosophila* larvae

Staphylococcus aureus ATCC 25923, a bacterial strain obtained from LGC Standards in Middlesex, UK, served as the bacteria model. The strain was stored at 4°C and cultivated individually in tryptic soy broth (TSB) media at 37°C. To define the bacterial infectious doses, the culture turbidity was measured using the colony-forming unit (CFU) method at 0.5 McFarland (approximately around 1.5×10^8 CFU/mL). The bacteria were subsequently collected, washed with phosphate-buffered saline (PBS), and utilized in the experimental tests. The infection experiment was carried out using two fly lines. For each fly line, as many as 200 of second instar *Drosophila* larvae were used for untreated control, and caffeine-treated groups (ten larvae per vial and five vials for each group). For the caffeine-treated groups, 150 larvae were divided into three groups (0.16, 0.8, and 4 mM) based on the previously described protocol [21,22], with slight modification. Briefly, the larvae were placed in a 1.5 mL microcentrifuge tube along with 500 µL of crushed bananas and 500 µL of an overnight bacterial culture. To ensure the larvae remained at the bottom of the tube throughout the infection, a foam stopper was used. Following this stage, the larvae were rapidly washed in PBS, transferred to a petri dish containing fly food that had been incorporated with caffeine, and subsequently incubated for 3 h at a temperature of 29°C.

Preparation of heat-killed *Escherichia coli* and autoinflammatory model using *Drosophila*

Heat-killed *Escherichia coli* (HKE) were obtained by autoclaving a freshly cultured *E. coli* culture for a duration of 30 min, based on the previously published protocol [23]. In this experiment, 400 of second instar larvae of the *PGRP-LB*$^{\Delta}$ line were selected and assigned into groups before transferring them into petri dishes containing fly food supplemented with heat-killed *E. coli* in addition to caffeine. These second instar *Drosophila* larvae were used for caffeine testing (two control groups and three treatment groups). For the treatment groups, 150 larvae were divided into three groups (ten larvae per vial and five vials for each group). A total of 100 larvae were divided into two control groups consisting of five replications per group. All fly groups were maintained at a temperature of 25°C.

Survival and locomotor assays

Survival assay was conducted based on the previously reported method [10,24] to examine the effect of caffeine administration on the lifespan of *D. melanogaster,* either wildtype or mutant lines. Adult wildtype flies that had been treated with caffeine were observed for their survival and documented every day. Adult wildtype flies that have been given the caffeine were also subjected to negative geotaxis-based locomotor assay, based on the previously reported method [18]. Briefly, *D. melanogaster* were placed into empty marked test vial equipped with a clear finish line. Before carrying out the test, vials were tapped to ensure that all *D. melanogaster* were at the bottom of the vials. The movements of flies were then observed for 15 seconds, and the numbers of flies that were able to cross the marked finish line were then counted.

Gene expression analysis

In this assay, ten larvae of *D. melanogaster* Oregon-R that had been treated with caffeine were then subjected to the RNA isolation procedure using the Pure Link RNA Mini Kit (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, USA), based on the manufacturer's instructions. Assigned larvae were collected separately and transferred to the allocated tubes. The levels of targeted genes were examined using the reverse transcriptase quantitative PCR (RT-qPCR) method. The RT-qPCR assay was carried out in a 10 µL reaction volume using the SuperScript III Platinum SYBR Green One-Step qRT-PCR (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, USA), according to the manufacturer's protocols. RT-qPCR run using a set of target gene primers (**Table 1**) were performed in a 10 µL reaction volume with detail as follows: one cycle of 37°C for 15 min followed by 95°C for 10 min was subsequently followed by 40 cyclic repeats of 95°C for 10 s, 63°C for 30 s and 72°C for 30 s. A standard melt curve analysis was carried out in each RT-qPCR run to verify that only the expected product had been amplified. The RNA level of host ribosomal protein gene (*rp49*) (used as an internal control) was examined by using one set of *rp49* primers (**Table 1**) using a similar protocol as the target genes.

Table 1. Primers used in the RT-qPCR assay

Genes	Forward primer	Reverse primer
Drs	5' – TTGTTCGCCCTCTTCGCTGCTCT – 3'	5' – GCATCCTTCGCACCAGCACTTAC – 3'
Attacin	5' – GATGGACGTGCTAATCTCTG – 3'	5' – GGCTTAGCCGAAATGATGAG – 3'
TotA	5'– CCAAAATGAATTCTTCAACTGCT –3'	5'– GAATAGCCCATGCATAGAGGA C – 3'
sod1	5' – AGGTCAACATCACCGACTCC – 3'	5' – GTTGACTTGCTCAGCTCGTG – 3'
sod2	5' – TGGCCACATCAACCACAC – 3'	5'– TTCCACTGCGACTCGATG – 3'
rp49	5' – GACGCTTCAAGGGACAGTATCTG – 3'	5'– AAACGCGGTTCTGCATGAG – 3'

Data analysis

Results from quantitative analyses are presented as the mean±SD from at least three independent experiments. For overall fly survival data, statistical analyses were performed using the log-rank test (Kaplan-Meier method). Gene expression data, expressed as Ct values, were processed with Rotor-Gene Q software. Subsequent statistical analysis was conducted using one-way ANOVA in GraphPad Prism 9 software (GraphPad Software Inc., California, USA). Additionally, one-way ANOVA was applied to analyze fly survival data at specific time points. In all analyses, a significance level of $p<0.05$ was considered statistically significant. The p-values are reported in the corresponding figures or figure legends, with any $p<0.05$ deemed significant.

Results

Caffeine was relatively safe for *Drosophila* larvae

In this study, we examined the anti-aging and immunomodulatory potentials of a compound with established pharmacological profiles and safety: caffeine. As a start, we examined whether this compound was safe for the *Drosophila* larvae. To do so, a simple *in vivo* toxicity assay was carried out, testing four different concentrations of caffeine on the *Drosophila* larvae. Caffeine at concentrations of 0.16–4 mM did not affect the growth of *Drosophila* larvae to develop into adults (**Figure 1**), suggesting that this compound was relatively safe for *Drosophila* larvae at the specified concentrations.

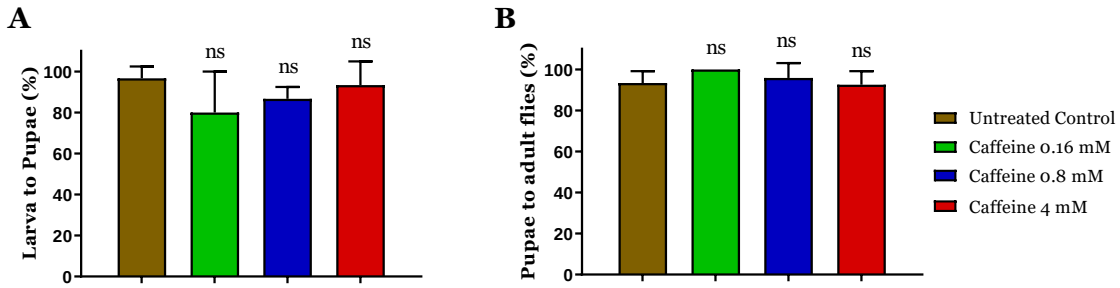

Figure 1. Safety assessment of caffeine in *Drosophila*. Caffeine is relatively safe for *Drosophila* larvae. Treatment of *Drosophila* larvae with caffeine at a range of 0.16 mM to 4 mM did not affect the developmental growth of *Drosophila* either at the larvae-to-pupae stage (A) or the pupae-to-adult stage (B).

Pretreatment of caffeine during larval stage improved the lifespan but not locomotor of *Drosophila*

With its relatively safe profiles in the *Drosophila* larvae, caffeine was then subjected to further analysis. *Drosophila* as a model can be used in phenotypical evaluations such as survival and locomotor assay on flies, either at larval or adult stages, upon the administration of drug candidates. Therefore, the effect of caffeine pretreatment was assessed separately on the lifespan and locomotor of flies, starting from larval stages until the end of their adult lifespan. Pretreatment of caffeine to *Drosophila* Oregon R larvae (until the larvae become pupae) resulted in the prolonged lifespan of adult *Drosophila* (**Figure 2A**).

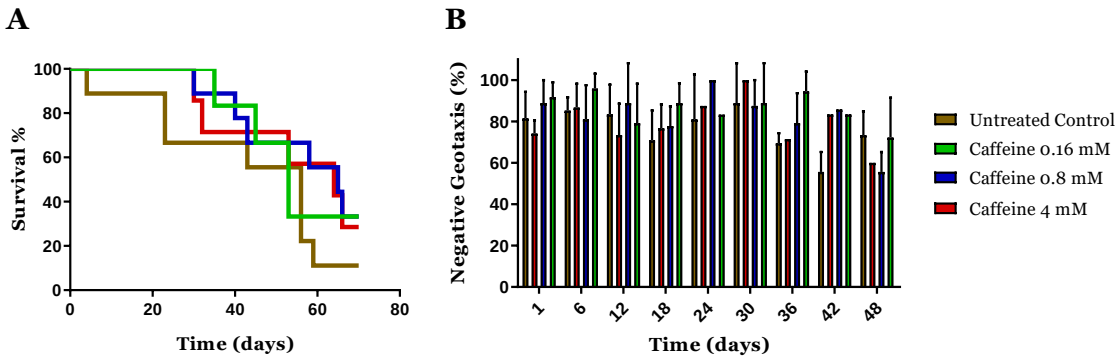

Figure 2. Effect of caffeine pretreatment in behavioral of *Drosophila*. Pretreatment of caffeine during larval stage resulted in the increased lifespan and locomotor improvement of adult *Drosophila* Oregon R. Lifespan improvement was observed in the group of flies pretreated with caffeine (A). In the negative geotaxis assay, *Drosophila* larvae pretreated caffeine demonstrated not significant improvement in the locomotor state of adult *Drosophila* (B). Each treatment was compared to the heat-killed *E. coli* control.

Improvement in the species survival can be linearly correlated with improvement in the locomotor activity. To examine this, a negative geotaxis assay was conducted on adult *Drosophila* that was successfully enclosed from the pupae contained in each group of flies. In this assay, flies that crossed the marked finish lane within 15 s were counted and the data was analyzed. The interpretation was groups with higher number of flies that can cross the marked finish lane were then regarded as groups with better locomotor phenotype. In accordance with that, a group of flies pretreated with caffeine (**Figure 2B**), demonstrated a non-significant number of flies that were able to cross the marked finish line, suggesting that caffeine consumption during the early life of *Drosophila* did not affect the locomotor activity.

Expression of the endogenous antioxidant genes upon caffeine treatment

Since caffeine pretreatment can improve the overall survival of flies, it was hypothesized that caffeine pretreatment may affect the transcriptional level of endogenous antioxidant genes, particularly the superoxide dismutases: *sod1* and *sod2*. To test this, transcriptional analysis using the RT-qPCR method was conducted. The results revealed that pretreatment with caffeine did not improve the expression of *sod1* and *sod2* (**Figure 3A-B**). Taken together, this data suggests that caffeine may act in a different fashion to improve the developmental growth and survival of the treated flies.

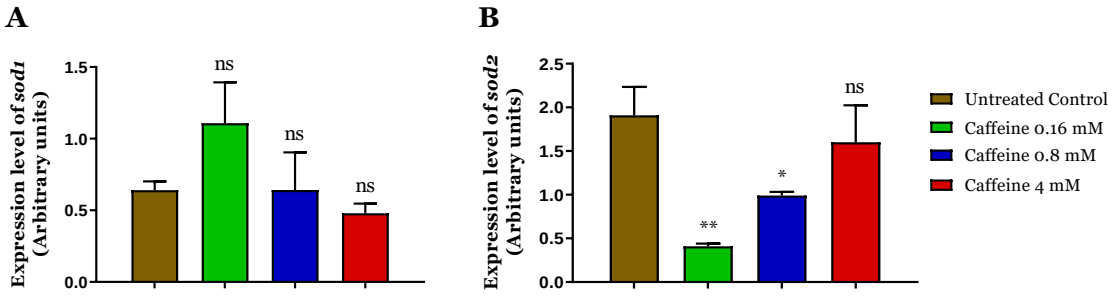

Figure 3. Transcriptional profiles of the *sod1* and *sod2* expression upon caffeine treatment in *Drosophila* larvae. Unchanged expression of *sod1* (A) and reduced expression of *sod2* (B) was observed in fly groups treated with caffeine at 0.8 and 0.16 mM, but not at 4 mM (A-B). ns: non-significant; * significant at $p<0.05$; ** significant at $p<0.01$.

Immunomodulatory effect of caffeine in the wildtype *Drosophila* larvae

To assess whether caffeine has high potential to be developed as an immunomodulator, real-time PCR-based molecular analysis on genes expressed was conducted under two different immune-related signaling pathways: NF-κB and JAK-STAT pathways. The results showed that flies pretreated with high concentration of caffeine (4 mM) exhibited low expression of *Drs* (a

signature gene commonly expressed under the regulation of the NF-κB-Toll pathway) (**Figure 4A**). Conversely, a low concentration of caffeine (0.16 mM) was able to induce the expression of *Atta* (a gene regulated by NF-κB-Imd pathway) in *Drosophila* larvae and such a profile was not observed in the ones pretreated with high concentrations of caffeine (**Figure 4B**). In addition, further assessment revealed the non-existent effect of caffeine on the expression of *TotA*, a JAK-STAT pathway target gene (**Figure 4C**).

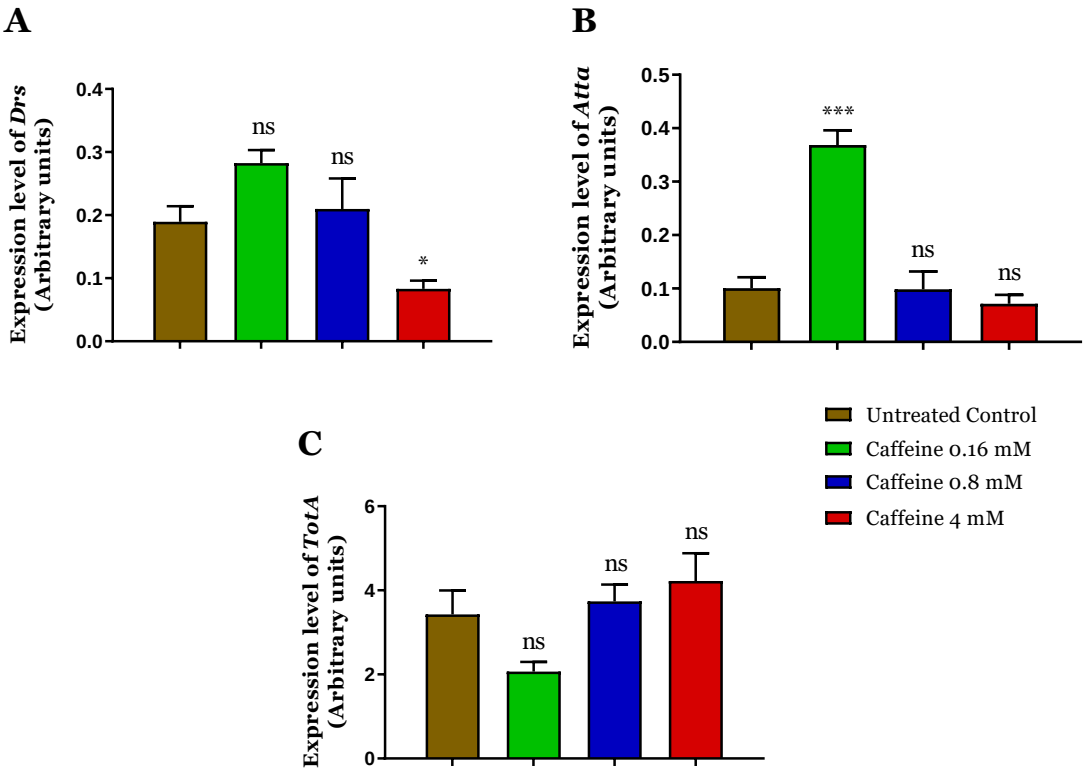

Figure 4. Transcriptional profiles of immune-related genes upon caffeine treatment in *Drosophila* larvae. Reduced expression of *Drs* was observed in the fly group treated with caffeine at 4 mM but not at lower concentrations (A). On the other hand, increased expression of *Atta* was evident in RNA samples prepared from flies treated with caffeine at 0.16 mM but not at higher concentrations (B). Caffeine, at any given concentrations, did not yield any effect on the expression of *TotA* (C). ns: non-significant; * significant at $p<0.05$; *** significant at $p<0.001$.

Immunosuppressive properties of caffeine in the *Drosophila* autoinflammatory model

In the previous results, we observed transcriptional changes in the immune-related genes upon the administration of caffeine in *Drosophila* larvae. To test whether this is relevant to the disease model, additional approaches were conducted to assess the immunomodulatory activities of caffeine by using two disease models: the *Drosophila* autoinflammatory model and the *Drosophila* infection model. In this study, HKE (heat-killed *E. coli*) was used as an NF-κB (Imd) pathway inducer to obtain the low survival state of *PGRP-LB*$^{\Delta}$ flies in the context of non-infection. Our data indicated that low survival of HKE-treated *PGRP-LB*$^{\Delta}$ flies was observed and this low survival state can be reversed upon the administration of fly food containing 0.16 and 0.8 mM of caffeine (**Figure 5A**), suggesting the possible attenuation of pro-inflammatory antimicrobial peptide expression. Subsequently, our objective was to elucidate whether the increased survival of *PGRP-LB*$^{\Delta}$ flies in the presence of caffeine concentrations could be associated with a reduction in the expression of antimicrobial peptides (AMPs). Among the AMPs expressed under the Imd (NF-κB) signaling pathway is Diptericin, encoded by the *Dpt* gene. Our analysis focused on assessing whether caffeine at these doses could impact the expression of the generated *Dpt* gene.

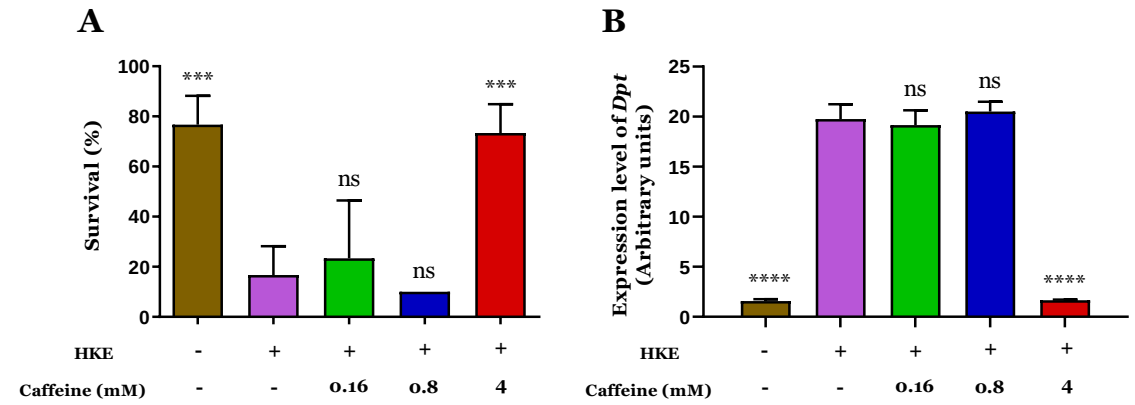

Figure 5. Effect of caffeine in the autoinflammatory fly model. Treatment of caffeine at higher concentrations but not at lower concentrations resulted in the increased survival of *PGRP-LB*$^{\Delta}$ larvae. Survival improvement was observed in the group of larvae treated with caffeine (A). Treatment of caffeine at higher concentrations suppresses the expression level of the *Dpt* gene in the *PGRP-LB*$^{\Delta}$ larvae (B). HKE: heat-killed *E. coli*; ns: non-significant; *** significant at $p<0.001$; **** significant at $p<0.0001$.

As depicted in **Figure 5B**, heat-killed *E. coli* induces the expression of *Dpt* in *PGRP-LB*$^{\Delta}$ flies, suggesting that heat-killed *E.* coli serves as an inducer of the Imd signaling pathway. It has been proven that overactivation of the Imd signaling pathway has been associated with reduced survival [25]. However, when heat-killed *E. coli* was co-administered with caffeine at concentrations of 4 mM, respectively, there was a decrease in the expression of genes related to NF-κB (*Dpt*) (**Figure 5B**). This reduction indicated an immunosuppressive effect, inhibiting the activation of the immune system, and leading to an increase in the survival rate of *PGRP-LB*$^{\Delta}$ (**Figure 5A**).

Non-antibacterial and immunomodulatory effect of caffeine in the *Drosophila* infection model

In the next attempt, an *in vivo* study was conducted to assess the immunomodulatory effect of caffeine by using the *Drosophila* infection model. In this experiment, *S. aureus* ATCC 25923 was used as the pathogen and the larvae of two different lines of *Drosophila*, Oregon R as wildtype (**Figure 6A**) and *Psh[1];;ModSP[KO]* as immunodeficient line (**Figure 6B**), as the object of infection. *Drosophila* larvae infected with *S. aureus* exhibited no significant survival improvement in the presence of caffeine (**Figure 6A**). Interestingly, low survival of *Psh[1];;ModSP[KO]* was detected in the presence of high concentration of caffeine (4 mM in fly food) **Figure 6B**).

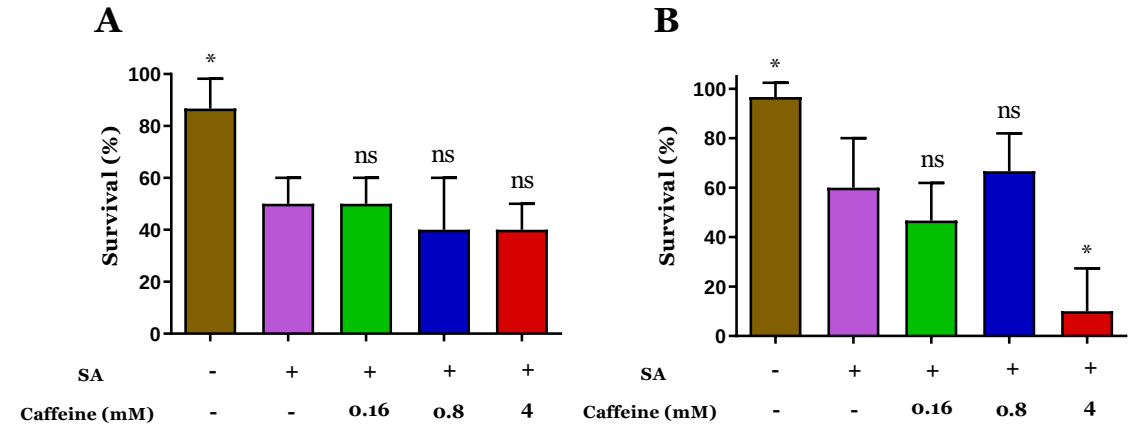

Figure 6. Effect of caffeine in wildtype and immunodeficient flies during SA infection. Caffeine treatment did not improve the survival of SA-infected *Oregon-R* (A) and SA-infected *psh[1];;modSP[KO]* mutant (B). HKE: heat-killed *E. coli*; ns: non-significant; SA: *Staphylococcus aureus*; * significant at $p<0.05$.

Discussion

We previously established an *in vivo* adult *Drosophila* platform to evaluate the immunomodulatory and anti-aging activities of drug candidates, successfully demonstrating the immunomodulatory effects of aspirin, dexamethasone, curcumin, and epigallocatechin gallate (EGCG) [10], along with the anti-aging properties of deoxycholic acid [11]. Despite the convenience of adult *Drosophila* in handling, its use necessitates anesthetic agents. Thus, to streamline the protocol and reduce costs associated with drug repurposing testing, we embarked on this study. Employing a *Drosophila* larval model in this study, we investigated caffeine's anti-aging and immunomodulatory potentials, building upon the principles from our prior work [10, 11]. We anticipate that utilizing the *Drosophila* larval model will yield rapid and comprehensive results in screening drug candidates with such properties. Our findings suggest caffeine's relative safety for *Drosophila* larvae and its ability to extend lifespan, albeit without significant locomotor improvements, supporting its potential anti-aging properties.

Several anti-aging indicators, including survival and locomotor activity enhancements, have been observed to correlate linearly with improvements in the cell redox state [18,25]. However, pretreatment with caffeine did not influence the expression of *sod1* and *sod2* (**Figure 3A-B**), suggesting a divergent mechanism for improving developmental growth and survival in treated flies. Notably, we did not assess global transcriptional changes in *Drosophila* upon caffeine treatment, which could provide further insights into its pharmacological activities on aging-related, developmental, and metabolic genes.

Given its genetic proximity to humans, *Drosophila* has emerged as a valuable model for drug discovery, particularly in immunology [6,9,23]. Equipped with a robust innate immune system comprising various anatomical barriers, immune cells, and signaling pathways [26,27], *Drosophila* offers insights into caffeine's immunopharmacological effects, potentially mediated through the NF-κB pathway.

Expanding upon our prior findings of caffeine-induced transcriptional changes in immune-related genes in *Drosophila* larvae, we assessed its immunomodulatory activities using two disease models: the *Drosophila* autoinflammatory model and the *Drosophila* infection model. For the autoinflammatory model, we used *PGRP-LB*$^{\Delta}$ mutant line. This line is characterized by the loss of PGRP-LB, a negative regulator of NF-κB (Imd) activation [28]. The loss of PGRP-LB plays a tremendous role in the overactivation of the NF-κB (Imd) pathway in the presence of a proper ligand, such as the cell wall component of Gram-negative bacteria, leading to the reduction in the survival rate of *Drosophila*. Based on our results, higher concentrations of caffeine demonstrated immunosuppressive properties by enhancing survival through suppression of NF-κB pathway.

Further experiment using the *S. aureus* infection model revealed that caffeine did not exhibit antibacterial or immunostimulatory activities, particularly evident using the immunodeficient *Psh[1];;ModSP[KO]* mutant line. The *Psh[1];;ModSP[KO]* mutant line is characterized by the absence of two crucial constituents, persephone (Psh) and modular serine protease (ModSP), within the Toll pathway. Both are important components required in the Toll pathway to generate antimicrobial peptides (AMPs), such as Drosomycin, which are crucial for the initiation of humoral innate immunity in reaction to SA infection in *Drosophila* [21,26]. In the absence of these two components, an immunodeficient-like state is induced, thereby simplifying the establishment of an infection model. *Drosophila* larvae infected with SA exhibited no significant survival improvement in the presence of caffeine (**Figure 6A-B**). This result suggested that caffeine might not possess antibacterial and immunostimulant activities at the tested concentrations. Interestingly, low survival of *Psh[1];;ModSP[KO]* was detected in the presence of high concentration of caffeine (4 mM in fly food). This is probably due to the strong immunosuppressive phenotype of caffeine at a concentration of 4 mM on the Imd pathway (see **Figure 5**). The Imd pathway has been shown to play a crucial role in regulating defense against gut infection caused by Gram-positive bacteria [27]. This case imposes a dual burden on the immune system (deficiency in both Toll and Imd pathways), leading to a significant decrease in the survival of SA-infected larvae.

Fruit flies have demonstrated utility as a model system for various human diseases. However, it is important to acknowledge that the *Drosophila* model, despite its value, possesses

less complex organ systems compared to mammals. Consequently, certain aspects of human physiology or disease processes involving complex organ interactions may not be faithfully represented in *Drosophila*. For instance, due to the absence of adaptive immunity in *Drosophila*, which plays a crucial role in the immune response of mammals, desired effects may not be observed. Therefore, further validation using mammalian models or human subjects is necessary.

Conclusions

In this research, we demonstrated the potential anti-aging and immunomodulatory effects of caffeine using *Drosophila* larval model, in agreement with the ones that have been reported in mammals [19,20]. Apart from the results obtained in this study, the *Drosophila* larval model may serve as an alternative *in vivo* drug testing platform with a high potential to yield reproducible results. In addition to the already established adult *Drosophila* model that we previously reported, our larval platform can offer a prompt, simple, and economical ways to screen drug candidates with anti-aging and immunomodulatory activities prior to further testing in higher mammalian model system. This approach will help to decrease the number of mammalian animals used in the pre-clinical *in vivo* study and, in time, will lower the overall cost of drug discovery. What is more, using this simple *in vivo* model system allows for simultaneous observation of possible adverse effects or even toxicity at the whole-body level. This model also offers the opportunity to investigate additional pharmacological effects (for example, antibacterial activity), thereby complementing the beneficial features offered by *in vitro* experiments.

Ethics approval

Not required.

Acknowledgments

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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