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Original Article

Dual effects of *Camellia sinensis* and *Andrographis paniculata* on hyperglycemia and infection in *Drosophila*

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Abstract

The coexistence of hyperglycemia and infectious diseases represents a critical global health challenge, particularly in resource-limited settings where it amplifies disease severity and complicates treatment approaches. Medicinal plants such as *Camellia sinensis* and *Andrographis paniculata* have gained recognition for their antioxidant, anti-inflammatory, and antimicrobial properties, making them promising candidates for addressing this double health burden. The aim of this study was to establish a preclinical model of hyperglycemia and infection (HI model) using *Drosophila melanogaster* and to investigate the therapeutic potential of *C. sinensis* and *A. paniculata* extracts in alleviating the burden associated with the HI condition. In this study, the HI model was established by simultaneously exposing *D. melanogaster* larvae to a high-concentration sucrose solution and *Staphylococcus aureus* for 24 hours. The larvae were then transferred to a high-sucrose diet supplemented with *C. sinensis* or *A. paniculata* extracts. Survival assays and molecular analyses were subsequently performed to evaluate the outcomes. Our findings revealed that the combination of hyperglycemia and infection significantly reduced survival rates in the *Drosophila* model. However, treatment with 1.25% *C. sinensis* and *A. paniculata* extracts notably improved survival, attributed to their antibacterial activity and regulation of key molecular pathways involved in immune responses, metabolic balance, and endogenous antioxidant defenses. These findings validate the utility of *D. melanogaster* as a model organism for investigating the double burden of HI. Furthermore, the study offers compelling evidence of the dual therapeutic potential of *C. sinensis* and *A. paniculata* in mitigating the detrimental effects of this condition. Overall, this research underscores the significant promise of plant-derived compounds in managing HI and paves the way for future studies to explore their underlying mechanisms and potential clinical applications.

Keywords: *Camellia sinensis*, *Andrographis paniculata*, hyperglycemia, infection, *Drosophila*

Introduction

The co-existence of metabolic disorders such as hyperglycemia with infectious diseases, termed the double burden, poses significant challenges to global healthcare systems [1]. Elevated blood glucose levels associated with hyperglycemia impair immune function, increasing vulnerability

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to infections and worsening their severity [2-4]. This dynamic creates a feedback loop, where hyperglycemia heightens the risk and impact of infections, while infections disrupt glucose metabolism, further complicating disease management [5]. Research has shown that hyperglycemia-induced immune dysregulation weakens the body's ability to defend against pathogens, resulting in prolonged infections and excessive inflammation [1]. These interactions significantly contribute to increased rates of mortality and morbidity, particularly in low-resource settings [1].

Antidiabetic drugs are widely used as a primary therapy to regulate blood glucose levels [6], prevent microvascular and macrovascular complications [7], and reduce patient care costs [8,9]. Additionally, various vitamins or supplements are often administered as supportive therapy [10]. In cases of infections in diabetic patients, antibiotics are typically prescribed [11]. However, it is important to note that managing diabetes complicated by infections often requires prolonged treatment, which can lead to polypharmacy [12]. Polypharmacy not only contributes to poor medication adherence but also increases the risk of drug interactions and adverse effects, particularly in elderly patients [13-15]. Furthermore, in infections arising as complications of diabetes, low patient compliance with antibiotic regimens may exacerbate the problem of antibiotic resistance [16]. These interconnected issues emphasize the critical need for more efficient therapeutic strategies.

Traditional medicinal plants represent a valuable source of bioactive compounds with potential dual pharmacological activities [17,18]. Traditional plants like *Camellia sinensis* (CS) [19-21] and *Andrographis paniculata* (AP) [22-24] have been shown to possess antibacterial and antihyperglycemic properties through various in silico, in vitro, and in vivo studies. As modern medicine evolves, the use of traditional plant-based remedies has gained popularity, especially for managing chronic conditions requiring prolonged treatment. This increased adoption is often linked to their efficacy and the relatively low incidence of adverse effects [25]. Nevertheless, a major global concern regarding the use of traditional medicinal plants is the insufficient scientific research and rigorous testing to substantiate their therapeutic benefits. Addressing this gap through comprehensive studies is crucial to unlocking their full pharmacological potential and ensuring their safe application in contemporary healthcare practices.

As an alternative model organism, *Drosophila melanogaster* can be effectively utilized in the screening of bioactive compounds and traditional medicinal plants [26-29]. Additionally, *D. melanogaster* has gained prominence as a powerful model for investigating various human diseases due to its highly conserved metabolic and immune pathways [30-33]. In recent years, *Drosophila* has been widely employed to study metabolic disorders, including hyperglycemia, providing valuable insights into disease mechanisms and potential therapeutic approaches [34], as well as immune responses to microbial infections [31,35,36]. Its short lifespan, genetic manipulability, and cost-effectiveness make *Drosophila* an ideal candidate for large-scale screening of therapeutic agents [32,37-41]. The high degree of conservation in insulin signaling [34], innate immunity [42], and endogenous antioxidant [43] genes between *Drosophila* and mammals support the use of this organism as a model for complex disease interactions, including the double burden of hyperglycemia and infection. Despite the well-established utility of *Drosophila* in modeling individual diseases, its application in investigating the interaction between metabolic dysfunction and infectious diseases remains limited, emphasizing the need for further research and translation to mammalian systems.

The aim of this study was to establish a double-burden model of hyperglycemia and infection (HI model) in *D. melanogaster* and to evaluate the protective effects of CS and AP extracts using the developed HI model. This study introduces a cost-efficient and robust preclinical screening model, particularly suited for application in developing countries such as Indonesia. The *Drosophila* HI model offers a promising in vivo platform for investigating hyperglycemia complicated by infection, enabling the rapid identification of natural compounds with dual therapeutic potential.

Methods

Study design, timeline, and setting

In this study, two *D. melanogaster* strains, w^{1118} and $psh^{1};;modSP^{KO}$, were utilized. These strains were obtained from the Laboratory of Host Defense and Responses at Kanazawa University, Japan. The w^{1118} strain, characterized by intact immune functionality, served as the primary model for survival analysis under hyperglycemic conditions, preliminary extract toxicity assessments, and molecular investigations. Additionally, this fly line was employed during the initial stages of establishing the HI model. The mutant strain $psh^{1};;modSP^{KO}$, deficient in two critical components of the Toll signaling pathway (Persephone and modSP), was specifically used to evaluate survival under infection-induced stress and to streamline the development of the HI model. The immunocompromised state of this mutant fly line facilitates the establishment of the infection model and allows for the concurrent assessment of antibacterial properties of candidate compounds [44]. All flies were maintained on standard fly food at 25°C for over 20 inbred generations before being used in the study to ensure genetic consistency.

The study commenced with initial survival assays using 96-well plates to independently evaluate the effects of hyperglycemia and bacterial infection on the two *D. melanogaster* lines (**Figure 1A-B**). Following the optimization of hyperglycemia and infection induction protocols, the HI model was developed using a similar method (**Figure 1B**). Notably, in this approach, a sucrose solution served as the sole nutritional source for the larvae; therefore, larvae maintained in wells containing 30% sucrose solution without bacterial exposure were used as controls to validate the HI model.

To assess the protective efficacy of extracts against the combined effects of hyperglycemia and infection, larvae from the HI model were transferred from the 96-well plates to a high-sucrose diet (HSD 30%) fly food supplemented with CS or AP extracts (**Figure 1C**), which had previously been confirmed to be safe through developmental toxicity assays. HSD 30% in fly food was used to sustain hyperglycemic conditions in larvae post-infection with *Staphylococcus aureus*. As a comparison, larvae from the control group, as described earlier, were transferred to standard fly food for further survival analysis.

Furthermore, gene expression levels were analyzed to explore molecular changes associated with hyperglycemia, bacterial infection, and extract treatments in the HI model. These analyses focused on genes involved in immune response (*drs*, *attaA*, and *totA*), metabolic regulation (*dilp2*), and endogenous antioxidant defense mechanisms (*sod1, sod2, cat,* and *gst*).

Induction of hyperglycemia and infection in *Drosophila* larvae

Hyperglycemia was induced using 60% sucrose (CAS No. 57–50–1, Smart Lab, Indonesia). *Staphylococcus aureus* ATCC 29213, obtained from LGC Standards (Middlesex, UK), was used as the infectious agent. The bacterial strain was stored at 4°C and cultivated on mannitol salt agar (MSA) medium at 37°C. The infectious dose was determined by measuring the culture turbidity, which was adjusted to approximately 6×10^{8} CFU/mL. The bacterial cells were harvested, washed with phosphate-buffered saline (PBS), and prepared for experimental use.

To develop the HI model, two *Drosophila* lines, w^{1118} and $psh^{1};;modSP^{KO}$ were used. A total of 384 second-instar larvae were used for each fly line, divided into four experimental groups (96 larvae per group), following a previously described protocol [45], with slight modifications. The experimental procedure involved the infection of *Drosophila* larvae with *S. aureus* in 96-well plates. Each well was prepared with 100 µL of 1.25% agarose in PBS. The bacterial suspension, prepared in a mixture of 60% sucrose solution and PBS, was added to the wells, followed by the transfer of a single larva using a fine paintbrush. Larvae treated with 30% sucrose solution in sterile PBS served as the control group. The 96-well microtiter plates were sealed with a breathable film, and four small holes were made in each well for ventilation. Plates were maintained at 25°C under a 12-hour light/12-hour dark cycle. This setup (**Figure 1B**) provides a controlled environment to establish a HI model in *Drosophila* larvae, facilitating the study of interactions between hyperglycemia and bacterial infection.

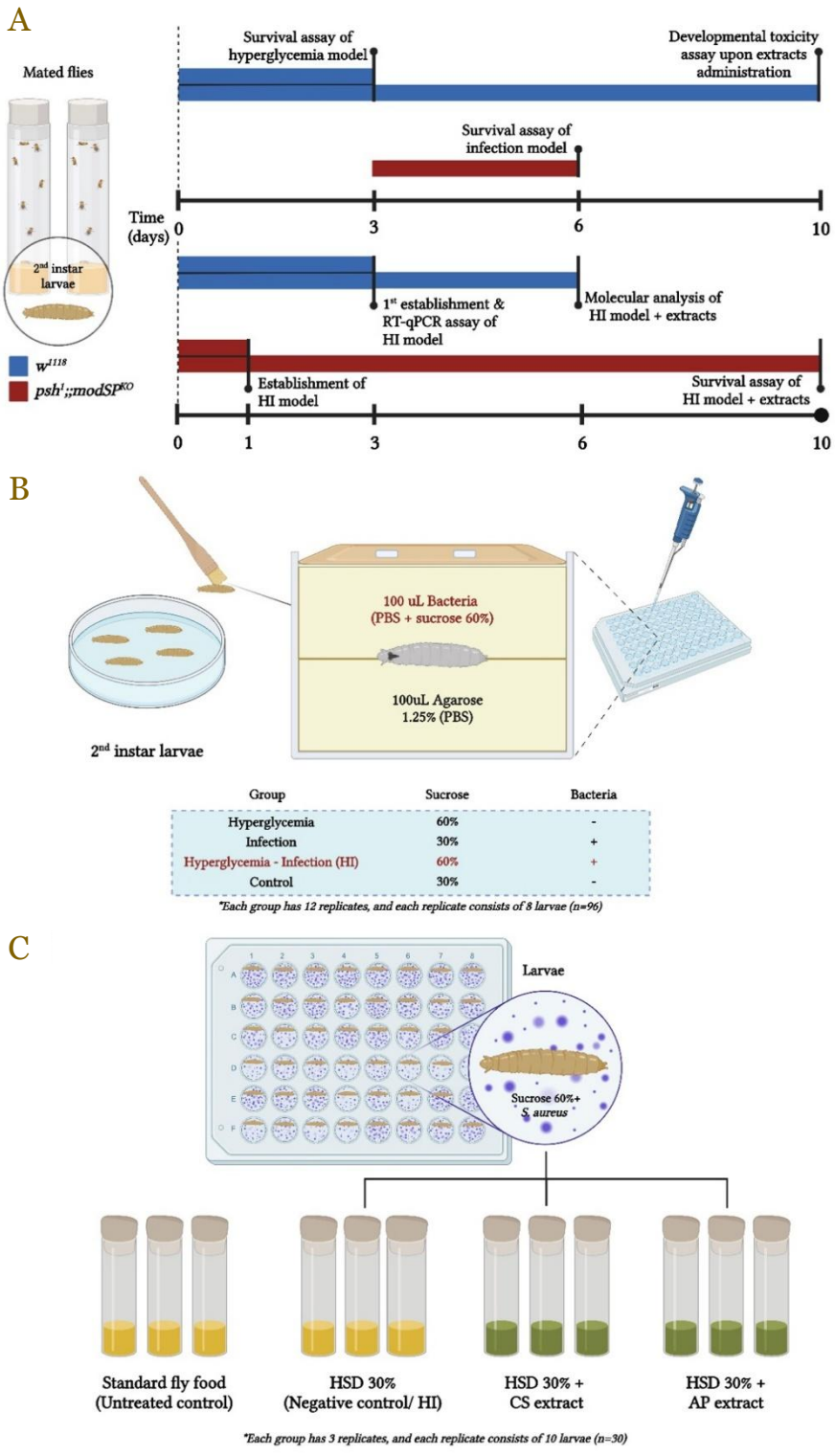

Figure 1. Study design, timeline, and settings. The research timeline consisted of key steps, including the establishment of the hyperglycemia and infection (HI) model, survival and developmental toxicity evaluations during extract administration, and molecular analysis (A). The establishment of the HI model was initiated by generating separated hyperglycemia and infection model groups, which were optimized to create the HI model using a 96-well plate (B). After the HI model was successfully developed, experiments were performed to evaluate the potential of *Camellia sinensis* (CS) and *Andrographis paniculata* (AP) extracts in mitigating the adverse effects of hyperglycemia and infection in this model. AP: *Andrographis paniculata*; CS: *Camellia sinensis*; HI: hyperglycemia and infection; HSD: high sugar diet; PBS: phosphate-buffered saline.

Preparation of extracts

Leaf samples of CS and AP plants were obtained from the Gunung Djenggot region, West Java, Indonesia, and Makassar, South Sulawesi Province, Indonesia, respectively. The plant specimens were subsequently authenticated at the Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Moslem University of Indonesia, Makassar. Following authentication, the samples were ground and passed through a No. 18 mesh sieve to obtain finely powdered CS and AP samples. Each 120 g of powdered CS and AP samples was subjected to maceration in 800 mL of 95% ethanol for five consecutive 24-hour periods. During maceration, the mixture was gently stirred every 24 hours to ensure effective extraction. After the maceration process, the liquid extracts were separated from the solid residues and concentrated using a rotary evaporator. The concentrated extracts were further dried on a water bath maintained at 50–60°C until a thick, semi-solid extract was obtained. This method ensured the efficient preparation of high-quality plant extracts for subsequent experimental applications.

Preparation of fly food containing extracts

The concentrated extracts, CS and AP, were first diluted in 70% ethanol to prepare stock solutions. These stock solutions were thoroughly mixed into standard cornmeal-based *Drosophila* fly food, resulting in final extract concentrations of 0.3125%, 0.625%, 1.25%, and 2.5% within the fly food. For experiments involving a high-sugar diet (HSD), the extracts were similarly incorporated into a modified fly food formulation containing 30% sucrose. This method ensured the uniform distribution of the extracts within the fly food, allowing for accurate assessment of their effects under both standard and high-sugar dietary conditions.

Survival assay

The survival assay was conducted to evaluate the effects of the burden (infection/hyperglycemia) and treatment on the survival of larvae. The survival assay was carried out based on a previously established protocol [45], with minor modifications. Larval survival was monitored for up to 24 hours following treatment. The number of larvae that survived exposure to bacteria, sucrose, or a combination of both, in the presence or absence of CS or AP treatment, was recorded for each condition. The survival data were analyzed and compared to the control group to evaluate the impact of the treatments.

Developmental toxicity assay

Following successful survival, the progression of larvae into pupae and subsequently into adult flies after treatment with fly food containing CS or AP was observed and recorded daily. Each treatment group was compared to the control, with three biological replicates per treatment, each consisting of ten larvae per vial.

Gene expression analysis

Total RNA was extracted from w^{1118} larvae using the PureLink RNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, USA), following the manufacturer's protocol. Gene expression analysis was conducted using Universal One-Step RT-qPCR Kit (Luna, New England Biolabs, Massachusetts, USA). Each RT-qPCR reaction was performed in a total volume of 10 µL and was carried out on a Gene Q Rotor platform (Qiagen, Germany). The protocol began with a reverse transcription step at 50°C for 10 minutes, followed by 40 cycles of PCR amplification. The PCR protocol included denaturation at 95°C for 15 seconds, annealing/elongation at 60°C for 30 seconds, and extension at 72°C for 30 seconds. To ensure the specificity of the amplified products, a melt curve analysis was performed across a temperature range of 60°C to 95°C. Primer sequences used for each target gene are listed in **Table 1**.

Table 1. List of primers used in RT-qPCR

Genes	Pathway	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
drs	Toll pathway (antimicrobial peptide)	TTGTTCGCCCTCTTCGCTGCTCT	GCATCCTTCGCACCAGCACTTAC
attaA	Imd pathway (antimicrobial peptide)	CCCGGAGTGAAGGATG	GTTGCTGTGCGTCAAG
totA	JAK/STAT	CCAAAATGAATTCTTCAACTGCT	GAATAGCCCATGCATAGAGGAC

Genes	Pathway	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
dilp2	Insulin-like peptide	TCTGCAGTGAAAAGCTCAACGA	CAAACTGCAGGGGATTGAGG
sod1	Endogenous antioxidant	AGGTCAACATCACCGACTCC	GTTGACTTGCTCAGCTCGTG
sod2	Endogenous antioxidant	TGGCCACATCAACCACAC	TTCCACTGCGACTCGATG
cat	Endogenous antioxidant	TTCCTGGATGAGATGTCGCACT	TTCTGGGTGTGAATGAAGCTGG
gst	Endogenous antioxidant	TCGAGTGGCCAAATTCGAGATCA	ACCACCTGTTCACATTGGCGTACT
rp49	Reference gene	CGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG

Data analysis

Survival and developmental toxicity data were analyzed using one-way ANOVA to compare differences between groups. Gene expression data, presented as Ct values, were initially processed using Rotor-Gene Q software (Qiagen, Germany) for data handling and normalization. Subsequent statistical analysis of gene expression and other datasets was conducted using one-way ANOVA in Prism® 9 software (GraphPad Software California, USA). A significance threshold of p=0.05 was applied to all analyses, with values below this threshold considered statistically significant.

Results

Establishment of hyperglycemia and infection (HI) model in *Drosophila* larvae

To facilitate the development of the HI model, preliminary screening was performed to evaluate the independent effects of hyperglycemia and infection on *Drosophila* larvae. Exposure to increasing sucrose concentrations resulted in a significant reduction in survival among w^{1118} larvae, with 60% sucrose determined as an appropriate baseline for inducing hyperglycemia under experimental conditions (**Figure 2A**). Similarly, $psh^{1};;modSP^{KO}$ larvae demonstrated a marked decrease in survival in response to escalating bacterial loads of *S. aureus* (**Figure 2B**). These results highlight the significant physiological burden imposed by hyperglycemia and infection individually, both of which critically diminish survival outcomes in *Drosophila* larvae.

In the establishment of the HI model, hyperglycemia and infection were concurrently induced, leading to a significant reduction in survival rates, which highlighted the synergistic effects of these conditions (**Figure 2C**). Molecular analysis revealed that hyperglycemia alone notably upregulated the expression of *dilp2* (**Figure 2D**), an insulin-like peptide gene, as part of a compensatory response to elevated glucose levels. However, when hyperglycemia was combined with infection, *dilp2* expression was significantly suppressed. Additionally, the expression of *drs* was significantly upregulated in response to *S. aureus* infection (**Figure 2E**). Hyperglycemia further enhanced *drs* expression under infectious conditions, suggesting an exacerbated immune response. These findings underscore the compounded physiological and immune challenges associated with the HI model, establishing it as a robust platform for investigating the complex interactions between hyperglycemia and infection.

Potential antibacterial activity of *C. sinensis* and *A. paniculata* extracts in the *Drosophila* HI model

Following the establishment of the HI model, the impacts of CS and AP extracts on survival within this model were evaluated. Prior to administering the extracts in the HI model, a preliminary toxicity assessment was conducted in w^{1118} larvae to confirm their safety during the developmental stages of *Drosophila* under normal conditions. The results of these observations are presented in **Figure 3**.

A concentration of 1.25% for both CS and AP extracts was identified as the highest level that remains safe for *Drosophila* development (**Figure 3A-D**). Using this concentration, the extracts were subsequently evaluated on larvae from the HI model. Notably, both the CS and AP extracts significantly improved larval survival (**Figure 3E**). The use of $psh^{1};;modSP^{KO}$ larvae in the HI model emphasized the potential antibacterial properties of these extracts. This antibacterial activity may contribute to their protective effects by reducing infection in the HI model. However, further investigations are required to elucidate additional mechanisms that may contribute to the overall protective effects of CS and AP extracts in this complex disease model.

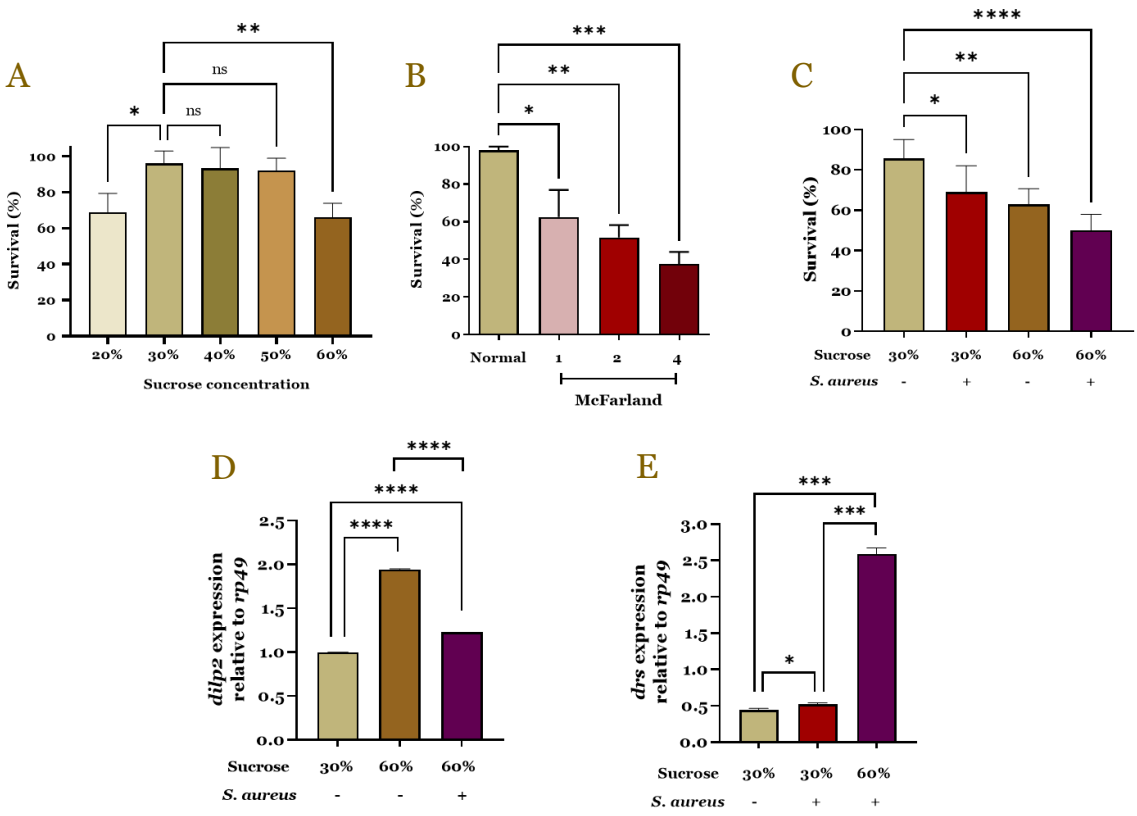

Figure 2. Impacts of elevated sucrose concentration and bacterial load on *Drosophila* survival. Increasing sucrose concentration (A) and bacterial load (B) lead to a reduction in the survival rate of *Drosophila* larvae. Meanwhile, simultaneous increases in sucrose concentration and bacterial load result in decreased survival of *Drosophila* (C). The impaired regulation of *dilp2* (D) and *drs* (E) is associated with reduced survival in the hyperglycemia and infection (HI) model. NS: non-significant; significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$.

Elevated expression of NF-kB and insulin-related genes in the *Drosophila* HI model upon *C. sinensis* and *A. paniculata* treatment

Previous analyses have shown that both CS and AP extracts possessed antibacterial properties, which enhanced survival in the HI model. However, we hypothesized that additional mechanisms are involved, particularly considering the nature of this study, which utilized a hyperglycemia and infection-induced HI model in immunodeficient larvae. Therefore, the effects of both extracts on immune and metabolic regulation were examined, as these factors may play a crucial role in survival outcomes. As presented in **Figure 4A-B**, both extracts significantly upregulated the expression of the *drs* gene, thereby enhancing innate immune defenses. Notably, the CS extract also increased the expression of the *attaA* gene, suggesting a broader immunomodulatory effect of CS compared to AP.

In contrast, the expression of the *totA* gene showed no significant change (**Figure 4C**), underscoring the predominant role of NF-κB-mediated pathways in this model. In terms of metabolism, both extracts elevated the expression of *dilp2* (**Figure 4D**), a gene associated with the insulin pathway, suggesting that CS and AP may restore energy homeostasis and improve insulin activity under the HI conditions. These results highlight the capacity of CS and AP to modulate both immune responses and metabolic imbalances, contributing to improved survival in the HI model.

Modulation of endogenous antioxidant activity through the administration of *C. sinensis* and *A. paniculata* in a *Drosophila* HI model

In addition to investigating the effects of CS and AP extracts on metabolic and immune-related gene expression, this study also investigated their influence on endogenous antioxidant activity in the HI model. The data indicated that CS extract significantly upregulated the expression of *gst* (encoding glutathione S-transferase) (**Figure 5A**), indicating an enhanced capacity for detoxifying reactive electrophilic species and mitigating oxidative stress. Conversely, AP extract

demonstrated a distinct effect by selectively increasing the expression of *sod* (encoding superoxide dismutase), specifically the *sod1* isoform (**Figure 5B**), but not *sod2* (**Figure 5C**), as well as *cat* (encoding catalase) (**Figure 5D**).

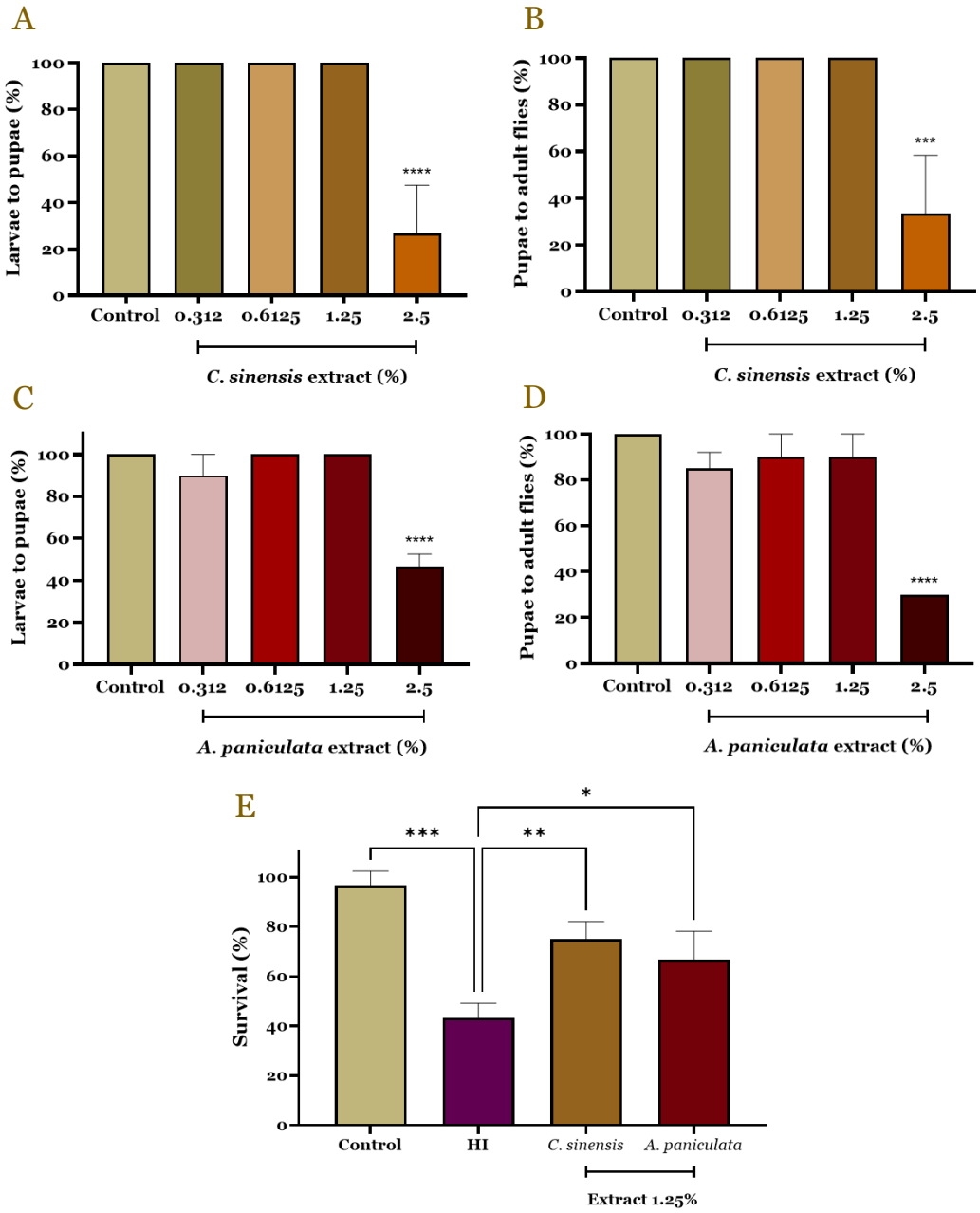

Figure 3. Effects of *Camellia sinensis* and *Andrographis paniculata* extracts administration on *Drosophila* larvae. Developmental progression from larvae to pupae (A, C) and pupae to adult flies (B, D) following treatment demonstrated that both extracts at 1.25% concentration were relatively safe and provided protective benefits, enhancing survival in the hyperglycemia and infection (HI) model. Significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$.

These findings suggest that AP extract targets specific oxidative radicals, such as superoxide and hydrogen peroxide, thereby reducing oxidative damage at the cellular level. These results suggest that both CS and AP extracts modulate oxidative stress pathways and mitigate oxidative damage at the cellular level. Their antioxidant activity emphasizes the protective role of these extracts in alleviating stress induced by hyperglycemia and infection in the *Drosophila* HI model, further supporting their potential as therapeutic agents for complex disease interactions.

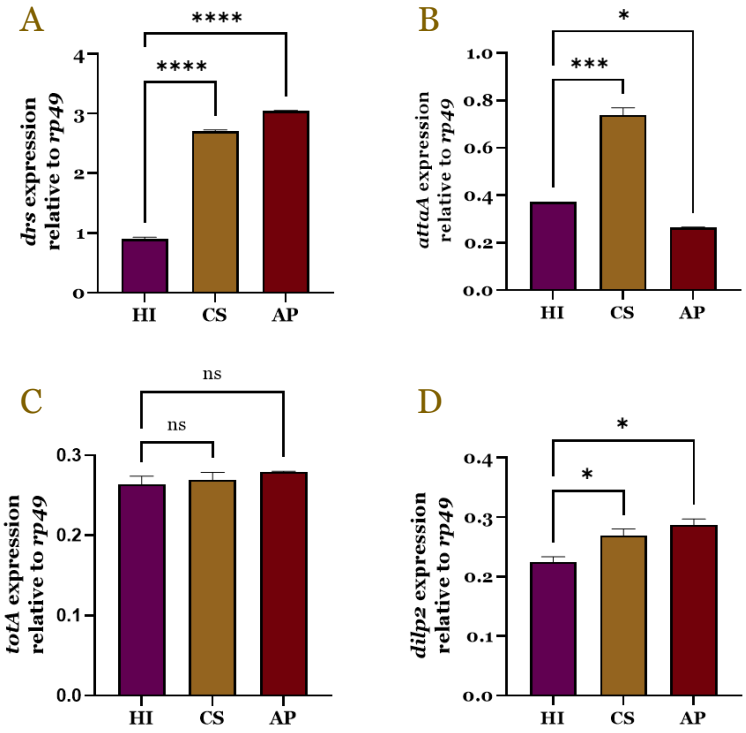

Figure 4. Administration of *Camellia sinensis* (CS) and *Andrographis paniculata* (AP) extracts positively modulated the interplay between immune and metabolic activities in the *Drosophila* model of hyperglycemia and infection (HI). Notable modulation was observed in the expression of NF-κB-dependent immune pathways (A-B), whereas the JAK/STAT pathway (C) remained unaffected. Furthermore, the extracts significantly impacted insulin-associated metabolic pathways (D), underscoring their dual role in enhancing immunity and metabolic regulation. NS: non-significant; significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$.

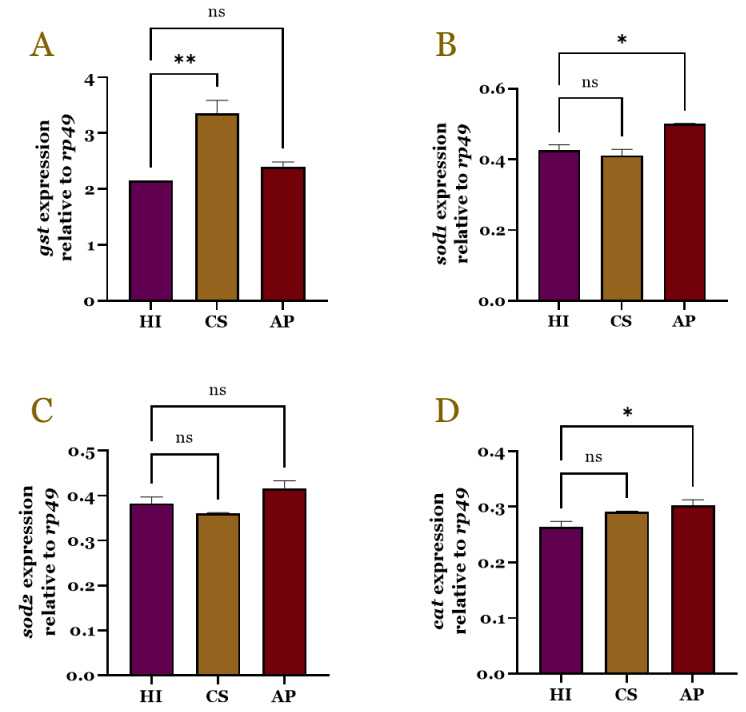

Figure 5. Modulation of endogenous antioxidant gene expression of *gst* (A), *sod1* (B), *sod2* (C), and *cat* (D) by *Camellia sinensis* (CS) and *Andrographis paniculata* (AP) extracts in the *Drosophila* model of hyperglycemia and infection (HI). *C. sinensis* extract promotes the upregulation of *gst* (A), while *A. paniculata* extract enhances the expression of *sod1* (B) and *cat* (D), emphasizing their potential to alleviate oxidative stress caused by hyperglycemia and infection. NS: non-significant; significant at $^{*}p<0.05$ and $^{**}p<0.01$.

Discussion

The increasing prevalence of diabetes complicated by co-infections underscores the need for an effective animal model that can simultaneously simulate hyperglycemia and infection, referred to as the HI model. This model provides a valuable platform for screening potential therapeutic compounds to address these intertwined conditions. In this study, we explored the therapeutic potential of Indonesian natural remedies, CS and AP extracts, using a *Drosophila*-based HI model. The study began with the establishment of the HI model in *D. melanogaster* by inducing hyperglycemia and bacterial infection concurrently. The results revealed that simultaneous increases in sucrose concentration and bacterial load significantly reduced larval survival (**Figure 2C**), highlighting the severe physiological burden imposed by the double burden condition. Molecular analysis revealed hallmark features of hyperglycemia and inflammation, with a notable decrease in *dilp2* expression (encoding *Drosophila* insulin-like peptide, a homolog of insulin in mammals) [46] and significant upregulation of *drs* expression (encoding Drosomycin, an essential antimicrobial peptide in the NF-κB pathway that targets Gram-positive bacteria) [42] (**Figure 2D-E**). These findings suggest an adaptive shift in metabolic energy toward immune responses under hyperglycemic and infectious stress. Consistent with previous studies, hyperglycemia was found to impair immune regulation, heightening vulnerability to infections [47].

After establishing the HI model, the effects of CS and AP extracts on *Drosophila* survival were evaluated at a previously shown to be safe concentration (1.25%). At this stage, the *psh*^{1}*;;modSP*^{KO} strain, a mutant deficient in critical components of the Toll signaling pathway (*psh* and *modSP*), was employed to confirm the antibacterial activity of both extracts. The absence of psh and modSP impairs the release of antimicrobial peptides, leading to an immunodeficient state that accelerates mortality following bacterial, especially Gram-positive infection [42,48,49]. Our data demonstrate the potential antibacterial activity of both CS and AP extracts, as evidenced by their significant improvement in larval survival in the HI model (**Figure 3E**).

Molecular analysis was conducted to assess the impact of CS and AP extracts on immunity and metabolism, previously shown to be influenced by hyperglycemia and infection in the HI model (**Figure 2**). In *Drosophila*, the Toll and Imd pathways regulate the innate immune response, with the Toll pathway primarily targeting Gram-positive bacteria and the Imd pathway addressing Gram-negative threats [42]. However, research by Hedengren *et al.* demonstrated that *S. aureus* infection activates both pathways, leading to antimicrobial peptide production [50]. The administration of CS and AP extracts enhanced the expression of *drs*, a key effector in the Toll pathway, suggesting their role in boosting innate immunity. Additionally, CS extract significantly upregulated the *attaA* gene (expressing Attacin A), mediated by the Imd pathway, indicating a broader immunomodulatory effect compared to AP. These results highlight the bioactive potential of CS and AP extracts in enhancing host defense against bacterial infections via modulation of innate immune pathways (**Figure 4A-B**). In contrast, the expression of the *totA* gene, which encodes Turandot A, an antimicrobial peptide regulated by the JAK/STAT pathway, showed no significant change (**Figure 4C**). The JAK/STAT signaling pathway has been shown to be essential for maintaining *Drosophila* body homeostasis during infection by regulating epithelial renewal processes through promoting cell proliferation in response to bacterial infections [51,52]. These findings confirm the protective effects of CS and AP through their antibacterial and immunostimulatory properties.

In addition to its effects on the immune system, this study also revealed that CS and AP extracts have the potential to improve energy metabolism disturbances commonly associated with HI. Our data indicated that treatment with CS and AP extracts resulted in an increased expression of *dilp2*, indicating the restoration of insulin sensitivity and improvement in energy homeostasis (**Figure 4D**). The catechins in CS extract are known to activate adenosine monophosphate (AMP)-activated protein kinase, an enzyme involved in enhancing insulin sensitivity and regulating energy metabolism [53]. Meanwhile, andrographolide in AP extract contributes by enhancing the function of pancreatic β-cells, thereby supporting insulin production and sensitivity [54]. The increased expression of *dilp2* suggests that both extracts work synergistically to address insulin release, one of the major complications frequently encountered in HI cases.

Oxidative stress, induced by hyperglycemia and infection, is one of the potential factors exacerbating the pathophysiology in cases of HI. Three key enzymes—superoxide dismutase, catalase, and glutathione peroxidase—are essential components of the antioxidant defense system, playing a critical role in protecting cells from oxidative damage [55]. Administration of CS extract has been shown to enhance the expression of *gst* that encodes the glutathione S-transferase, an enzyme involved in the detoxification of free radicals through the glutathione system [56]. This finding indicates the potential of CS to increase cellular antioxidant capacity, primarily supported by its polyphenol content, especially catechins, which exhibit potent antioxidant properties [57]. In addition, AP extract demonstrated a specific effect by upregulating the expression of *cat* that encodes catalase, an enzyme that decomposes hydrogen peroxide into water and oxygen [58]. This mechanism highlights the potential role of AP extracts in eliminating specific oxidative radicals, such as hydrogen peroxide, which might help prevent cellular damage. Furthermore, the expression of other antioxidant genes, such as *sod1* but not *sod2*, also showed a significant increase, reinforcing the broader and more potent antioxidant effects in counteracting free radicals generated by hyperglycemia and infection.

This study underscores that CS and AP extracts have complementary mechanisms in addressing the conditions associated with double burden-hyperglycemia and infection (**Figure 6**). Both extracts have similar patterns in modulating the immune system, metabolism, and antioxidant capacity, albeit through different pathways. CS extract is more effective in activating the innate immune response by increasing the expression of *drs* and *attaA* genes, while AP extract is more prominent in reducing oxidative stress through the upregulation of *cat* and *sod1* genes. The observed synergistic effects of both extracts indicate their potential to collectively enhance immune function, restore metabolic homeostasis, and mitigate oxidative stress. This synergy highlights the promise of CS and AP as integrated therapeutic agents for HI. Furthermore, their therapeutic potential extends beyond addressing the metabolic and infectious complications associated with HI, presenting opportunities for the development of more comprehensive treatment strategies. This approach aims to concurrently modulate immune dysfunction, energy metabolism, and oxidative stress, thereby offering a multifaceted therapeutic intervention for managing complex disease pathophysiology.

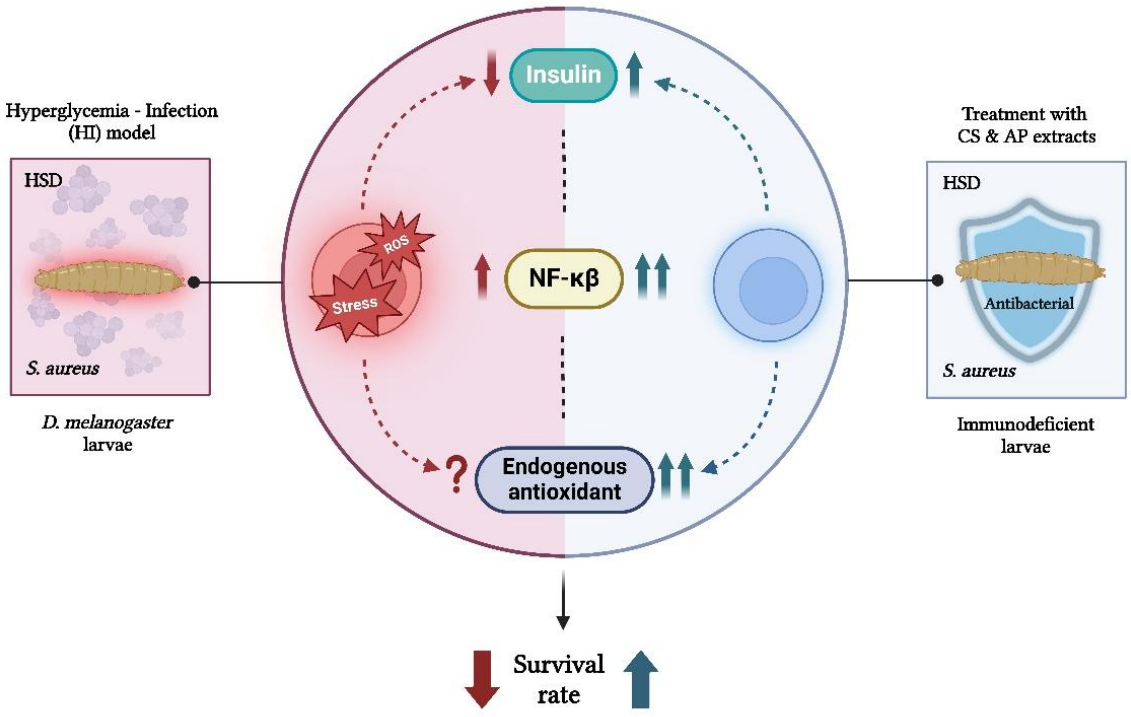

Figure 6. Potential protective mechanisms of *Camellia sinensis* (CS) and *Andrographis paniculata* (AP) in *the Drosophila* model of hyperglycemia and infection (HI).

Conclusion

In this study, an HI model combining hyperglycemia and infection in *D. melanogaster* was successfully established to explore the physiological and molecular effects of these coexisting conditions. The coexistence of both conditions in the *Drosophila* model led to a significant reduction in survival rates. The administration of *C. sinensis* and *A. paniculata* extracts exhibited notable protective effects, as reflected in enhanced survival rates, attributed to their antibacterial activity, positive effects on modulating immune responses, and improvements in metabolic and antioxidant functions. These findings emphasize the potential of *C. sinensis* and *A. paniculata* as promising natural remedies for addressing the multifaceted challenges of the HI model. This study highlights the significance of incorporating bioactive natural compounds into therapeutic approaches for managing complex diseases related to hyperglycemia and infection.

Ethics approval

Not required.

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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.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies during the manuscript preparation. AI-based language model ChatGPT was employed in the language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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