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Synergistic Antihyperglycemic Effects of Soursop Leaves and Garlic in Alloxan-Induced Rats

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A R T I C L E I N F O

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A B S T R A C T

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin production. Natural remedies, such as soursop leaves (Annona muricata) (SL) and garlic (Allium sativum) (GAR), are known for their antihyperglycemic properties. This study aimed to investigate the effects of SL and GAR extracts, individually and in combination, on reducing blood glucose levels in alloxan-induced diabetic rats. A total of 24 male Wistar rats were divided into six groups, receiving treatments of either metformin, SL, GAR, or their combination for seven days following alloxan administration to induce hyperglycemia. Blood glucose levels were measured before and after induction, and post-treatment on day 15. The results showed that the combined extracts exhibited better antihyperglycemic activity compared to individual treatments, with the most effective dose being 26.3 mg/kg BW SL + 31.5 mg/kg BW GAR.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, which can lead to severe complications if not properly managed. Natural remedies, particularly medicinal plants, have gained increasing attention as complementary therapies for diabetes due to their bioactive compounds. Soursop (Annona muricata) leaves and garlic (Allium sativum) are known for their potential health benefits, including antioxidant, anti-inflammatory, and glucose-lowering properties. Previous studies suggest that the combination of multiple plant-based treatments may produce synergistic effects, enhancing their therapeutic potential. This study explores the antihyperglycemic effects of combining soursop leaves and garlic in a model of alloxan-induced diabetic rats, aiming to uncover new insights into plant-based interventions for hyperglycemia management.

The effectiveness of natural remedies often depends on the optimal dosage and combination of bioactive components to achieve desired therapeutic outcomes. In the case of soursop leaves (SL) and garlic (GAR), both contain compounds such as alkaloids, flavonoids, and organosulfur elements that contribute to glucose regulation and antioxidant activity. The combined extract is expected to produce a synergistic antihyperglycemic effect by complementing each other's mechanisms, such as enhancing insulin sensitivity, reducing oxidative stress, and modulating glucose metabolism. Determining the appropriate doses of the SL and GAR combination is crucial, as it ensures maximum efficacy while minimizing potential side effects. Varying doses are often tested to identify the most effective concentration capable of significantly reducing blood glucose levels in alloxan-induced diabetic rats, paving the way for more refined applications in natural diabetes management.

Male rats were chosen as experimental subjects to ensure consistency in the results, as hormonal fluctuations in female rats could introduce variability, particularly in glucose metabolism and insulin sensitivity. The weight of the animals is also carefully monitored, as it plays a critical role in both baseline metabolic activity and the development of diabetes following alloxan induction. Maintaining uniform weight across the subjects ensures that differences in the outcomes are due to the treatment rather than variations in body mass. Additionally, stress is a key factor to consider in animal studies, as it can influence glucose levels through the release of stress hormones like cortisol, potentially confounding the results. Efforts are made to minimize stress through proper animal handling, acclimatization, and controlled environmental conditions to ensure that the observed antihyperglycemic effects of the combined soursop leaf and garlic extract reflect the treatment's true efficacy, rather than being impacted by external stressors.

Monitoring blood glucose levels (mg/dl) before and after treatment is essential to evaluate the effectiveness of the combined soursop leaves and garlic extract in managing hyperglycemia. Baseline glucose levels are measured prior to the induction of diabetes with alloxan, providing a reference point for the animal's normal metabolic state. Following alloxan administration, a significant

increase in blood glucose levels is expected, confirming the onset of hyperglycemia and the successful establishment of the diabetic model. The treatment phase involves administering varying doses of the SL and GAR combination, and glucose levels are measured at specific intervals to track changes over time. A decrease in blood glucose levels post-treatment would indicate the antihyperglycemic potential of the extract, suggesting improved glucose regulation. This comparison between pre- and post-treatment glucose levels provides crucial data for assessing the extract's efficacy and determining the optimal dose needed to achieve significant glycemic control.

Despite advances in the management of diabetes mellitus (DM), it remains a chronic condition characterized by persistent hyperglycemia due to impaired insulin production (IDF, 2021; Lauralee, 2010). While Indonesia is rich in medicinal plants with therapeutic potential, such as soursop leaves (Annona muricata) (SL) and garlic (Allium sativum) (GAR), their combined effects on blood glucose regulation remain underexplored. Both SL and GAR contain bioactive compounds like flavonoids, which improve insulin sensitivity, reduce oxidative stress, and protect pancreatic beta cells from damage (Dewi et al., 2020; Prihanti et al., 2019). Although previous research has shown that 300 mg/kg BW of SL enhances pancreatic islet cells, and GAR has shown promise as a supplement for DM management (Dewi et al., 2020), the synergistic potential of these two plants when combined remains uninvestigated. Given the growing interest in multi-herbal treatments to enhance efficacy through synergistic mechanisms (Balkrishna et al., 2024), there is a critical gap in determining whether the combination of SL and GAR extracts could offer enhanced antihyperglycemic effects. Furthermore, while prior studies have explored individual extracts of SL and GAR, the impact of their combination on cognitive function in alloxan-induced diabetic models has yet to be evaluated (Sanie-Jahromi et al., 2023; Yedjou et al., 2023; Yunivita et al., 2019). This research aims to address these gaps by investigating the anti-diabetic properties and potential cognitive benefits of SL and GAR extracts, both individually and in combination, as an alternative therapeutic approach.

The purpose of this study is to investigate the anti-diabetic effects and potential cognitive enhancement benefits of soursop leaves (SL) and garlic (GAR) extracts, both individually and in combination, as alternative therapies for alloxan-induced diabetic conditions. Specifically, the research aims to evaluate the ability of these extracts to lower blood glucose levels and improve cognitive function compared to the standard antidiabetic drug metformin. By comparing the outcomes between the alloxan-induced group, metformin-treated group, and the extract-treated groups, the study seeks to identify any significant differences in their therapeutic efficacy.

LITERATURE REVIEW

The management of diabetes mellitus (DM), a chronic metabolic disorder, increasingly includes exploring natural remedies, particularly due to the bioactive compounds found in medicinal plants. Among these, soursop leaves (*Annona muricata*) and garlic (*Allium sativum*) have demonstrated potential

benefits, including glucose-lowering, antioxidant, and anti-inflammatory properties. Research suggests that combining multiple plant-based treatments may create synergistic effects, where each component enhances the effectiveness of the other, providing greater therapeutic impact. For instance, soursop leaves contain flavonoids and alkaloids that support pancreatic cell health and glucose metabolism, while garlic contributes organosulfur compounds that boost insulin sensitivity and reduce oxidative stress. This study explores the potential synergistic antihyperglycemic effects of combined soursop leaf and garlic extracts in alloxan-induced diabetic rats, aiming to identify whether this herbal combination can effectively reduce blood glucose levels and improve cognitive functions. Through this approach, the research seeks to uncover a holistic, plant-based alternative that could complement existing diabetes treatments and add valuable insights into natural diabetes management strategies.

The following is the framework in this study:

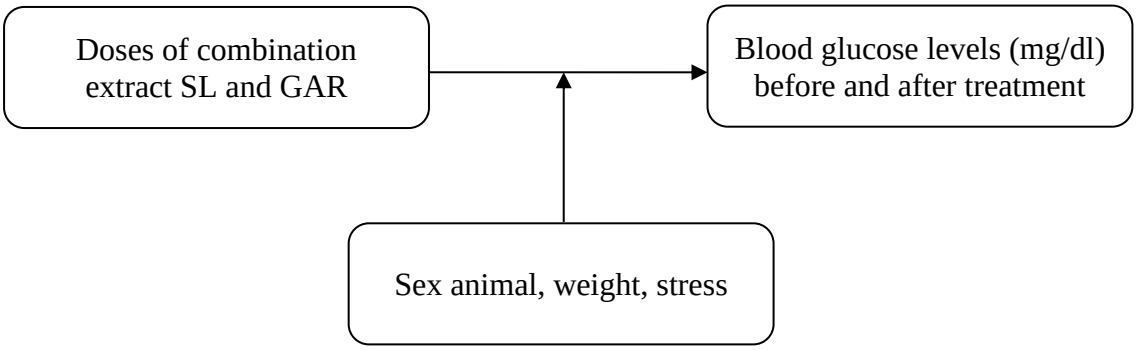

Figure 1. Conceptual Framework

METHODOLOGY

Male Wistar albino rats weighing 150–250 g were obtained from the Palembang Rat Centre, Indonesia, and acclimatized for one week under controlled conditions, maintaining a 12:12 hour light-dark cycle, temperatures between 24°C to 26°C, and humidity levels of 60% to 65%. The animals were given unrestricted access to food throughout the study. All procedures followed the Animal Act of 1986 (scientific procedures) and were approved by the Ethics Committee of the Faculty of Medicine, Universitas Lampung, with approval number KET-1600/UN26.18/PP.05.02.00/2023.

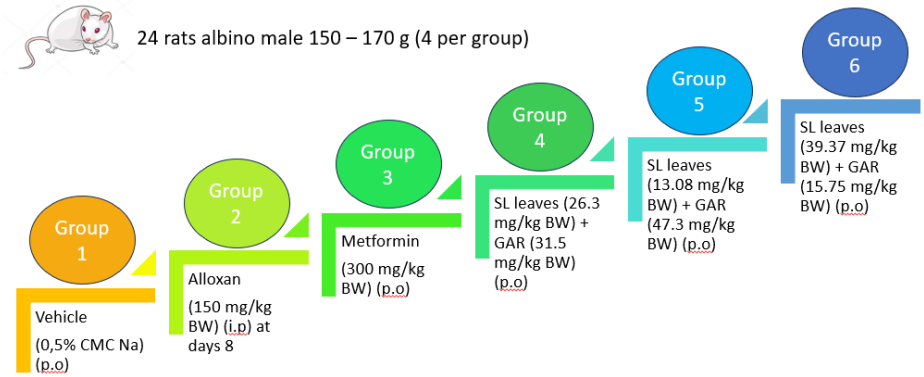

Figure 2. Design Anima Experimental

The dried powders of soursop leaves (SL) and garlic (GAR) were sourced from Tambahrejo, Pringsewu Regency, Lampung Province, Indonesia, and their taxonomic verification was confirmed at the Department of Biology, Mathematics, and Life Science, Universitas Lampung. Five hundred grams of each powder were macerated with 96% ethanol (Merck, Germany) for 24 hours, followed by filtration. The filtrates were concentrated using a rotary vacuum evaporator (Heidolph, Schwabach, Germany) at 40°C to produce solvent-free extracts. The final extracts were weighed, and the percentage yield was calculated to ensure extract quality and consistency.

The experiment involved 24 Wistar rats randomly divided into six groups, with four rats per group. Alloxan at a dose of 150 mg/kg BW (Ighodaro et al., 2017) was used to induce hyperglycemia, and metformin served as the reference antidiabetic drug. The ethanolic extracts of SL and GAR, along with metformin, were administered at a dose of 300 mg/kg BW based on prior studies (Zhou et al., 2024; Jayanti Djrami et al., 2023; Marpaung, 2020), all suspended in 0.5% CMC-Na and given daily for seven days. On day 15, blood glucose levels were measured using a glucose meter kit. Blood samples were collected through fingertip punctures, with alternative sites such as the forearm or palm suggested to minimize discomfort (Topping et al., 2019). Statistical analysis was conducted using GraphPad Prisms 10, applying one-way and two-way ANOVA followed by Tukey's post-hoc test. Results were expressed as mean ± SEM, with p-values <0.05 and <0.01 considered statistically significant.

RESULT AND DISCUSSION

Blood glucose levels were measured on day 15 using a glucose meter kit to evaluate the antidiabetic effects of the treatments. Following alloxan induction to elevate glucose levels, the rats were treated with metformin for one week. Subsequently, the ethanolic extracts of soursop leaves (SL) and garlic (GAR), either individually or in combination, were administered daily for another week. Figures 2A and 2B display the blood glucose levels before and after alloxan induction, while the glucose levels following the treatment period are presented in Figure 1D.

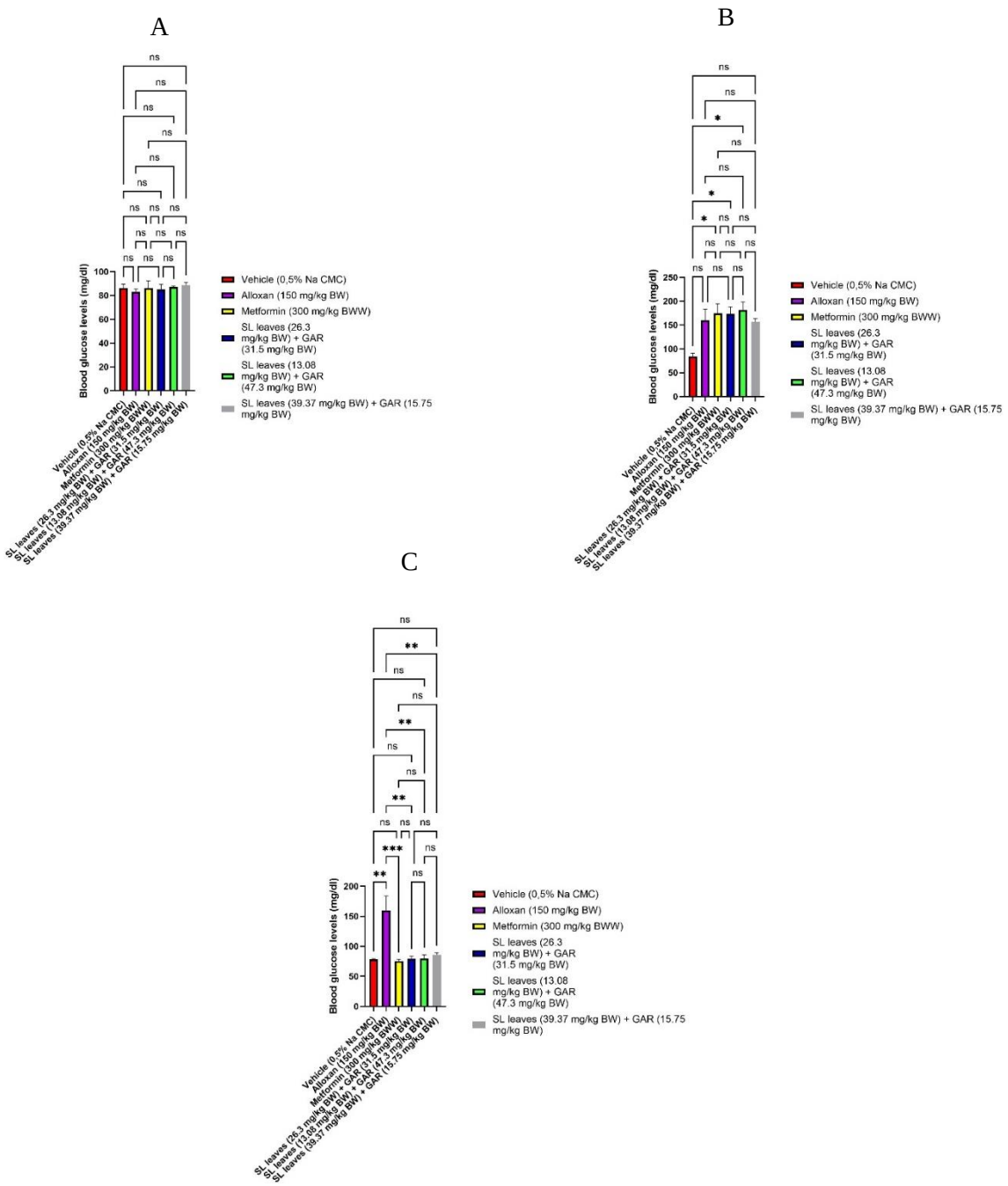

Figure 3. Blood Gluose Levels. A. Before Induction. B. After Induction. C. After Treatment. All Data Expressed Means ± SEM, N = 4. *P < 0.05, **P < 0.01, ***P < 0.001, Ns = Not Significance

The data in Figure 3 offer insights into the effects of alloxan induction, metformin treatment, and the administration of soursop leaves (SL) and garlic (GAR) extracts, either individually or in combination, on blood glucose levels. The figure is divided into three panels: A shows the baseline blood glucose levels before alloxan induction, B presents the levels immediately after alloxan

induction, and C displays the glucose levels after the treatment period with extracts and metformin.

In Figure 3A, the results confirm that, before induction, blood glucose levels were comparable across all groups, with no significant differences observed, as indicated by the non-significant (ns) p-values. This establishes that the animals started the study under similar physiological conditions, providing a reliable baseline for comparison.

Figure 3B shows the blood glucose levels after alloxan administration, where a significant increase in glucose levels is observed in the alloxan group compared to the control group (vehicle). This demonstrates the successful induction of hyperglycemia. Additionally, while metformin administration resulted in a partial reduction in glucose levels compared to the untreated alloxan group, the effects of the SL and GAR extracts, especially in combination, show a noticeable downward trend, though some combinations only achieve marginal statistical significance (*$p < 0.05$).

In Figure 3C, the post-treatment data reveal that metformin and certain combinations of SL and GAR extracts effectively lowered blood glucose levels. Specifically, combinations with higher SL doses (e.g., 33.37 mg/kg BW SL + 15.75 mg/kg BW GAR) show significant improvements, indicated by p-values < 0.05 and < 0.01. This supports the hypothesis that the combined extracts exhibit synergistic effects in lowering glucose levels. While some lower doses were less effective, the trend suggests that the combination of SL and GAR provides an enhanced antidiabetic effect, possibly due to the complementary mechanisms of action, such as insulin sensitization and oxidative stress reduction.

CONCLUSION AND RECOMMENDATION

This study demonstrated that soursop leaves (SL) and garlic (GAR) extracts effectively reduce blood glucose levels in alloxan-induced diabetic rats, with the combination of both extracts showing a greater antihyperglycemic effect than individual treatments. Notably, the dose combination of SL leaves at 26.3 mg/kg BW and GAR at 31.5 mg/kg BW exhibited the most promising results, suggesting a synergistic interaction between the two extracts. These findings highlight the potential of SL and GAR as alternative therapies for diabetes management. Further research is recommended to isolate and identify the active compounds responsible for this synergistic effect and to assess the impact of the combination on pancreatic cell function in animal models.

ADVANCED RESEARCH

These findings suggest a synergistic effect between the two extracts in managing hyperglycemia. Further research is recommended to identify the active compounds within the combined extracts and evaluate their impact on pancreatic cell regeneration. This study underscores the potential of soursop leaf (SL) and garlic (GAR) extracts as alternative or complementary therapies for diabetes management.

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