Thamar University Journal of Natural & Applied Sciences 11(1) (2026) 34– 39

DOI: https://doi.org/10.59167/tj3pbg80

TuJNaS

Original Article

Preliminary Evaluation of Acute Toxicity and Hypoglycemic Effects of *Salvia officinalis* L. Extract in Healthy Male Rabbits: An Animal Model Study

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Received: 9 December 2025. Revised: 2 April 2026. Accepted: 3 April 2026. Published: 29 June 2026.

Abstract

Background: *Salvia officinalis L.* is an aromatic perennial herb belonging to the Lamiaceae family, known for its diverse pharmacological properties, including potent antioxidant, antimicrobial, anti-inflammatory, neuroprotective, and anti-diabetic activities. Despite its extensive traditional use, the specific roles of *S. officinalis* in glucose management and its acute toxicity profile in rabbits require further elucidation. **Objective:** This study provided a preliminary assessment of the acute toxicity profile and the basal glycemic effects of a methanolic extract of *S. officinalis* in a normoglycemic rabbit model. **Methodology:** Twelve adult male White rabbits were randomly assigned to four groups (n=3). Group I served as the vehicle control, while Groups II, III, and IV received single oral doses of *S. officinalis* extract at 1000, 1500, and 2000 mg/kg, respectively. Animals were monitored over 14 days for toxicity, mortality, and body weight, alongside repeated measurements of fasting blood glucose (FBG). Data were analyzed using two-way repeated measures ANOVA. **Results:** *S. officinalis* extract demonstrated dose-dependent basal glucose modulation. The repeated measures analysis revealed highly significant effects for both time and dose ($P < 0.001$). The 1000 mg/kg dose was identified as the No Observed Adverse Effect Level (NOAEL), while the 1500 mg/kg dose was established as the Maximum Tolerated Dose (MTD). At 2000 mg/kg, an observed lethal threshold was recorded with a 66.7% mortality rate. **Conclusion:** The preliminary findings suggest that *S. officinalis* extract exhibits potential dose-dependent hypoglycemic activity in normoglycemic models. However, the manifestation of systemic toxicity at higher concentrations defines a narrow therapeutic window for the extract.

Keywords: *Salvia officinalis*; Hypoglycemic; Acute Toxicity; Rabbit Model; Safety Assessment

1. Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1, 2]. Globally, DM prevalence poses a significant public health challenge, with estimates of 537 million adults affected, expected to rise to 643 million by 2030 and 783 million by 2045. The increasing resistance to standard therapies and treatment costs has led to a renewed interest in complementary and alternative medicine, particularly traditional herbal remedies for DM management [3–5].

In this context, *Salvia officinalis* (common sage or garden sage), an aromatic perennial herb belonging to the Lamiaceae family, has garnered significant scientific attention [6]. Historically recognized for its diverse pharmacological spectrum, this herb exhibits potent antioxidant, antimicrobial, anti-inflammatory, neuroprotective, and notably, anti-diabetic activities [7]. The therapeutic potential of *S. officinalis* is primarily attributed to its complex phytochemical composition, which includes essential oils, flavonoids, and phenolic acids. These constituents are thought to exert their hypoglycemic effects through various mechanisms, such as improving insulin sensitivity, enhancing glucose uptake, and inhibiting α-glucosidase activity [8–15]

Despite the extensive traditional application and a growing body of in vitro and in vivo literature elucidating its general pharmacological effects, several critical aspects regarding the safe and effective use of *S. officinalis* in DM management remain inadequately addressed. Specifically, there is a notable lack of robust experimental evidence defining the acute safety profile (NOAEL, MTD) and the precise dose-response relationship of its methanolic extract. Therefore, this study aims to provide a preliminary characterization of the acute safety profile and explore the basal glycemic effects of *Salvia officinalis* methanolic extract in a normoglycemic rabbit model, establishing a pharmacological baseline for future larger-scale investigations.

2. Methodology

2. 1 Plant Material and Extract Preparation

Fresh leaves of *Salvia officinalis L.* were collected in February 2025 from a house garden in Dhamar city, Yemen. The leaves were formally identified and authenticated by Dr. Abdullah Al-Shawsh, Department of Botany, Thamar University, Yemen, Under voucher specimen (No. TU+AC-2024). Certificates of authentication were subsequently submitted and retained in the Department of Pharmacy, Thamar University, Yemen.

The collected *S. officinalis* leaves (Figure 1) were thoroughly washed with distilled water and shade-dried at ambient temperature [11]. The dried leaves were subsequently pulverized into a fine powder using a mortar and pestle, according to established methods [11, 16]. A methanolic extract was then prepared via maceration. To ensure a sufficient yield for high-dose administration, the extraction was conducted in successive cycles (10 g of powder per 100 mL solvent) for 48 hours with occasional agitation. The filtrate was concentrated using a rotary evaporator at 40 °C under reduced pressure. The crude methanolic extract was stored in airtight amber vials at 20 °C until further use, following standard protocols [4, 17, 18]. Finally, the total yield of the extract was determined [19].

2. 2 Experimental Animals

A total of twelve adult male White rabbits (1000–1500 g, 18 weeks old) were sourced from the local market. The animals were housed in metallic cages at the Thamar University Institute for Continuous Education. All procedures involving the animals adhered to the internationally recognized guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee (AEC) of Thamar University [20, 21].

Figure 1: Leaves of the *Salvia officinalis*.

Rabbits were acclimated for one week under controlled conditions (23 ± 2 °C, 55 ± 5% humidity, 12:12 h light/dark cycle) [22] [23]. Following acclimatization, animals were randomly assigned to four groups (n = 3 per group) for the **Preliminary Acute Toxicity and Dose-Ranging Assessment** (Table 1). The use of four parallel groups with n=3 was adopted as a **preliminary screening method** to establish the **Maximum Tolerated Dose (MTD)**. Group I (Control): Received vehicle only (distilled water), Group II-IV: Received single oral doses of sage extract at 1000, 1500, 2000 mg/kg, respectively. The extract and vehicle were administered as a single oral dose (P.O.) via oral gavage

Table 1: Design and distribution of experimental animals grouping.

Group	Category	Dose Administration
Group I	Vehicle control	Distilled water (vehicle)
Group II	Low-Dose Toxicity/Efficacy	Extract 1000 mg/kg BW (p.o.)
Group III	Mid-Dose Toxicity/Efficacy	Extract 1500 mg/kg BW (p.o.)
Group IV	High-Dose Toxicity/Efficacy	Extract 2000 mg/kg BW (p.o.)

Note: The *S. officinalis* extract was administered as a single oral dose (P.O.) via oral gavage. Animals were fasted overnight before dosing to stabilize baseline glucose.

2. 3 Preliminary Acute Toxicity Assessment and Body Weight

The acute oral toxicity was assessed using a dose-range finding study (limit test) to characterize the initial safety profile of the extract. In accordance with the 3Rs principles for animal reduction and the exploratory nature of this screening, the No Observed Adverse Effect Level (NOAEL) and Maximum Tolerated Dose (MTD) were identified based on the highest doses resulting in zero mortality. Clinical signs were monitored using the Functional Observational Battery at least twice daily. Observations for behaviour, water/food intake, and mortality were recorded over a period of 14 days. This preliminary screening focused on clinical and observational parameters, including mortality and physical indicators. Body weights were measured using an electrical balance at baseline Day 0 and at the end of the experiment (Day 14). Body weight data were statistically analyzed and expressed as mean ±SEM. Figure 2 shows a graphical representation of the study methodology.

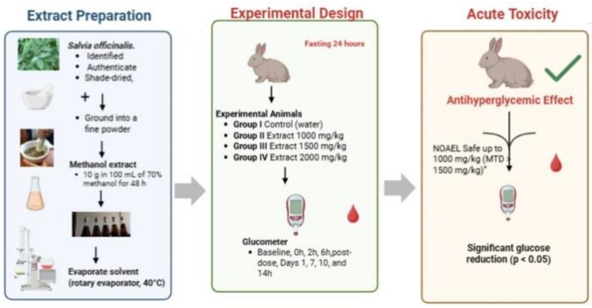

Figure 2: Graphical representation of *Salvia officinalis* ethanolic extract is safe and demonstrates significant antihyperglycemic activity in rabbits.

2. 4 Assessment of Effect on Basal Blood Glucose Levels

To evaluate the hypoglycemic efficacy of the *S. officinalis* extract, fasting blood glucose (FBG) levels were monitored in normoglycemic rabbits. Baseline measurements (0 h) were obtained immediately prior to extract administration. Post-treatment glucose levels were then recorded at acute intervals (0, 2, and 6 h) and sustained intervals (Days 1, 7, 10, and 14). Blood samples (~3mL) were collected from the marginal ear vein via lancet puncture, and glucose concentrations were determined using a validated portable glucometer (Accu-Chek, Roche Diagnostics, Germany) in accordance with the manufacturer's instructions.

2. 5 Ethical Approval

All experimental procedures were conducted in accordance with the ethical guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee (AEC) of Thamar University (**Ref No: TU-2025-045**). Animals were monitored every 2 hours during the first 24 hours for clinical signs of toxicity. Humane endpoints were established to prevent unnecessary suffering; animals showing severe distress or moribund conditions were slated for immediate euthanasia using an overdose of anesthesia. Rabbits that succumbed to the high-dose toxicity (2000 mg/kg) were recorded, while surviving healthy animals were maintained under veterinary supervision for the 14-day observation period before being humanely retired from the study.

2. 6 Statistical Analysis.

Data were expressed as mean ± SEM and analyzed using SPSS software (version 16). For body weight data, differences among groups at Day 14 were analyzed by One-way ANOVA. For fasting blood glucose data, a **two-way repeated measures ANOVA** was employed to evaluate the main effects of Dose and Time, as well as their interaction. **Bonferroni post-hoc tests** were performed to identify significant differences between groups at specific time points. Mortality rates were compared by Fisher's exact test. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

3. Results

3. 1 Plant Material and Extract Yield

The extraction process, involving a 48-hour maceration of 10 g of dried *S. officinalis* leaves in 70% methanol, resulted in a dark brown, gummy crude extract. The calculated percentage yield was 10.3% (w/w). This yield provided a sufficient quantity of the phytochemical constituents required for the subsequent acute toxicity and hypoglycemic assessments in the rabbit model.

3. 2 Preliminary Acute Toxicity and Clinical Observations

Oral administration of the methanolic extract of *S. officinalis* resulted in dose-dependent mortality and pronounced clinical manifestations (Table 2). All rabbits (3/3) in Group II survived the 14-day observation period, establishing this dosage as the NOAEL. Conversely, Group III recorded a 33.3% mortality rate (1/3), while Group IV exhibited the highest fatalities at 66.7% (2/3). These findings identify 1500 mg/kg as the MTD and approximately 2000 mg/kg as the observed lethal threshold.

Clinical signs also followed a dose-dependent pattern. While the control and Group II exhibited normal behavior and stable vital signs, rabbits receiving 1500 and 2000 mg/kg displayed pronounced neurobehavioral alterations, including drowsiness, trembling, and aggressiveness. Severe manifestations, such as paralysis and asphyxiation, were exclusively recorded in Group IV, reflecting acute systemic toxicity.

Table 2. Acute Toxicity and Clinical Observations of *S. officinalis* Methanolic Extract in Male Rabbits Over 14 Days

Mortality and clinical signs	Group I (Control)	*S. officinalis* methanolic extract		
		Group II (1000 mg)	Group III (1500 mg)	Group IV (High 2000 mg)
Mortality	0/3	0/3	1/3	2/3
Behavioral Changes	None	None	drowsiness, trembling,	Aggressiveness, paralysis, drowsiness, trembling, asphyxiation
Body Temperature	Normal	Normal	Normal	Elevated
Heart Rate	Normal	Increased	Increased	Increased
Respiratory Rate	Normal	Slightly Increased	Increased	Increased

***Note:** N: normal, AN: abnormal, P: presence, A: aggressive.*

3. 3 Food and Water Consumption

As shown in Figures 3A and 3B, no statistically significant differences in mean food and water intake were observed across all treated groups compared to the control (P > 0.05). These results suggest that the extract did not impact appetite or hydration, indicating that observed weight changes were likely driven by metabolic or toxicological factors rather than reduced intake.

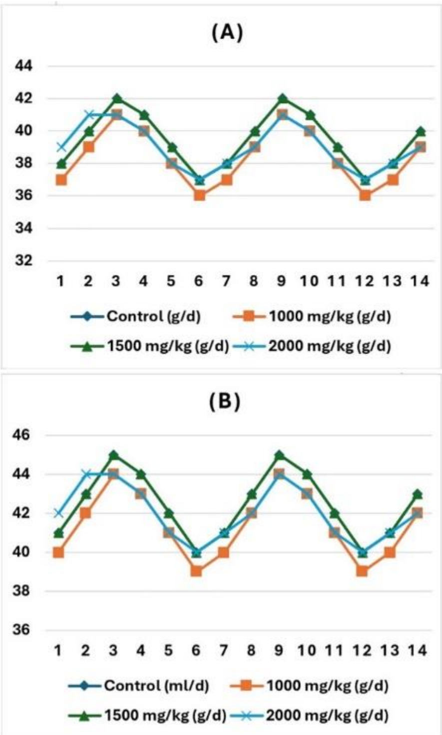

Figure 3: Food consumption expressed in g/d **(A)** and Water intake expressed in ml/d **(B)** of male rabbits orally administered with *S. officinalis* extract.

3. 4 The effect of Salvia officinalis extract on Rabbit's body weight

As demonstrated in Table 3, rabbits in the vehicle control group (Group I) exhibited a statistically significant increase in body weight from 1300 ± 20 g on day 0 to 1395 ± 25 g on day 14 (P < 0.001). In the Low-Dose Group II (1000 mg/kg), a marginal yet statistically insignificant increase in body weight was noted between day 0 and day 14 (P > 0.05). Conversely, the Mid-Dose Group (Group III) (1500mg/kg) exhibited a statistically significant reduction in body weight (P < 0.001), with a mean difference of −160±10, suggesting an early indication of systemic toxicity. In contrast to the control and low-dose groups, the High-Dose Group (Group IV) (2000 mg/kg BW) demonstrated the most severe and statistically significant reduction in body weight, dropping from 1302 ±21 g on day 0 to 1120±25 g on day 14 (P <0.001), strongly correlating with the observed mortality.

Table 3: The mean body weight and weight difference of male rabbits on day 0 and day 14.

Groups	Body weight on day 0 (g)	Body weight on day 14 (g)	Mean difference ± SEM (g)	P value
Group I	1300±20	1395±25	+95±5	<0.001
Group II	1305±22	1320±27	+15±5	>0.05
Group III	1310±20	1150±30	- 160±10	<0.001
Group IV	1302±21	1120±25	- 182±6	<0.001

***Note:** Data are expressed as mean ± SEM (n=3). The initial body weight range for all rabbits was 1000–1500 g. P-values compare Day 14 weight to Day 0 weight within the same group.*

3. 5 Hypoglycemic Efficacy and Basal Glucose Modulation

As summarized in Table 4, the methanolic extract exhibited a potent dose-dependent hypoglycemic effect across all treated groups compared to the control. In the Low-Dose Group II (1000 mg/kg), a significant reduction in FBG was observed at 2- and 6-hours post-administration (P < 0.05) compared to its baseline. The most pronounced reduction was recorded in the High-Dose Group IV (2000 mg/kg), where blood glucose levels dropped significantly from a baseline of 168.00 ± 1.36 mg/dL to a minimum of 60.00 ± 1.32 mg/dL at 2 hours (P < 0.001).

As illustrated in the longitudinal profile (Figure 4), the extract demonstrated a rapid onset of action within the first 6 hours, followed by a gradual stabilization phase. While all treated groups maintained lower FBG levels relative to their respective baselines through Day 14, the efficacy of the high-dose group was confounded by the previously noted systemic toxicity and mortality rate.

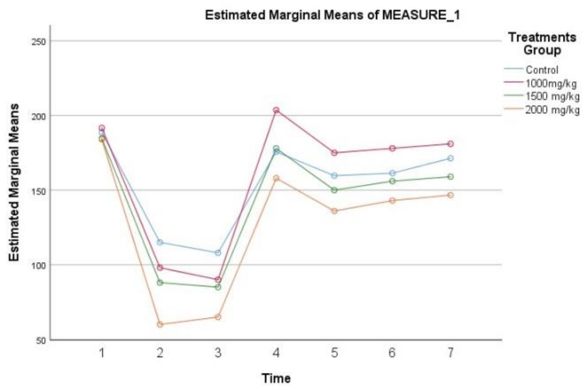

Figure 4: Longitudinal glycemic profile of normoglycemic rabbits following acute oral administration of *Salvia officinalis* L. extract. The graph depicts the dose-dependent reduction in fasting blood glucose (FBG) during acute (0–12 h) and sustained (1–14 days) observation periods. Error bars indicate SE.

4. Discussion

Medicinal plants have demonstrated significant utility within the realm of traditional medicine and hold relevance in economically disadvantaged nations [24–26]. The present study provides a preliminary characterization of the acute toxicity profile and the basal glycemic effects of a methanolic extract of *Salvia officinalis* in rabbits. By establishing the No Observed Adverse Effect Level (NOAEL) and the Maximum Tolerated Dose (MTD), the results yield preliminary insights into the safety threshold and metabolic response in a normoglycemic state associated with this botanical. Our findings revealed that the methanolic extract of *S. officinalis* diminishes blood glucose concentrations, especially at elevated dosages, while exhibiting a dose-dependent toxicity profile.

Table 4: Fasting blood glucose levels (mean ± SEM) in male rabbits following acute oral administration of *S. officinalis* extract.

Group	Treatments	Blood glucose (mg/dl)						
		0	2^h	6^h	1^{ST} Day	7^{TH} Day	10^{TH} Day	14^{TH} Day
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Group I	Control Group	188±2.88 ^{A}	115±3.00 ^{A}	108±2.65 ^{A}	175±2.08 ^{A}	159±0.58 ^{A}	161±1.53 ^{A}	171±2.31^{A}
Group II	1000 mg/kg	191±2.87 ^{A}	98±2.00 ^{B}	90±2.00 ^{B}	203±3.22 ^{A}	175±3.0 ^{B}	178±3.0 ^{B}	181±3.0 ^{B}
Group III	1500 mg/kg	184±4.50 ^{A}	88±2.00 ^{C}	85±2.00 ^{B}	178±1.0 ^{A}	150±2.0 ^{C}	156±3.0 ^{A}	159±3.0 ^{C}
Group IV	2000 mg/kg	168±3.60 ^{A}	60±2.00 ^{D}	65±2.00 ^{C}	158±1.0 ^{A}	136±2.0 ^{D}	143±3.0 ^{C}	146±3.06 ^{D}
P ± value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note*: Data are expressed as Mean ± SEM (Standard Error of the Mean), with n=3 per group. Values within the same column followed by different superscript letters (a, b, c, d) are significantly different at p < 0.05, as determined by a two-way repeated measures ANOVA followed by a Bonferroni post-hoc test for multiple comparisons.*

The preliminary assessment of acute toxicity revealed a clear dose-dependent relationship between extract administration and systemic safety. While the 1000 mg/kg dose was well-tolerated, the emergence of neurobehavioral alterations and mortality at 1500 and 2000 mg/kg defines a narrow therapeutic window for the methanolic extract in this animal model. The observed clinical signs, including trembling and paralysis at high doses, suggest potential neurotoxic or systemic metabolic distress when safe thresholds are exceeded. Crucially, the significant reduction in body weight observed in Groups III and IV (P < 0.001) must be interpreted as a primary indicator of systemic toxicity. This finding implies an estimated MTD of approximately 1500 mg/kg, reflecting the extract's potential for dose-dependent toxicity [11] and reaffirming its safety profile at moderated dosages [7, 11]. This specific delineation of the MTD in male rabbits, alongside comprehensive clinical observations over 14 days, constitutes a critical contribution to the toxicological data of *S. officinalis*. While food and water intake remained stable, the rapid weight loss strongly correlated with the high mortality rate and clinical deterioration. This suggests that the extract, at high concentrations, may interfere with metabolic homeostasis or induce acute organ stress, highlighting the critical importance of dose optimization to avoid adverse outcomes.

Beyond the evaluation of toxicity, the present study illustrates that the methanolic extract of *Salvia officinalis* effectively lowers blood glucose levels in rabbits. Notably, the high-dose cohort (2000 mg/kg) exhibited a significant reduction in fasting blood glucose levels, a finding that is consistent with previous research highlighting the antidiabetic potential of *Salvia officinalis*. For example, previous studies [27] reported significant decreases in glucose levels in alloxan-induced diabetic rabbits subsequent to the administration of a 500 mg/kg dose of *Salvia officinalis* extract [28, 29]. The efficacy observed with the high-dose treatment in our investigation underscores the role of *Salvia officinalis* as a potent hypoglycemic agent in this model. However, the lack of a consistent dose-dependent response at elevated concentrations necessitates further exploration. It is conceivable that the intricate pharmacokinetics of the extract, encompassing absorption rates and metabolic pathways in healthy rabbits, may influence its efficacy across varying dosages.

Beyond the evaluation of phytochemical constituents, previous studies have indicated that the leaves of common sage contain elevated levels of flavonoids and saponins, among other phytochemicals [30]. The proposition that hypoglycemic activity may stem from the individual or synergistic effects of these compounds is well-documented [17, 31]. Furthermore, *S. miltiorrhiza* (Chinese sage) possesses a metabolite profile analogous to that of *S. officinalis*. Consequently, the observed declines in blood glucose levels in our study can be attributed to multiple potential mechanisms. Phytochemical analyses have identified flavonoids and saponins as key constituents known to exhibit antidiabetic properties; these may enhance insulin sensitivity and stimulate insulin secretion while concurrently diminishing hepatic glucose production [28, 32].

Additionally, the antioxidative characteristics of *Salvia officinalis* may mitigate oxidative stress, thereby safeguarding pancreatic beta-cells and sustaining insulin synthesis. Subsequent investigations delineating the specific pathways influenced by *S. officinalis* extracts could yield profound insights into its mechanistic underpinnings [33].

The findings of the current investigation are congruent with research conducted on diabetic models, such as [34], which indicated that the protective efficacy of *S. officinalis* extracts against weight reduction is contingent upon dosage and metabolic condition. The recorded weight decline at 1500 mg/kg and 2000 mg/kg in our healthy rabbits is plausibly attributable to the acute systemic stress and toxicological manifestations observed at these concentrations. This potentially culminates in the augmented degradation of structural proteins and muscular tissue, as previously elucidated within the framework of exacerbated catabolism [34, 35].

The significant reduction in fasting blood glucose observed in this study aligns with findings reported in other normoglycemic (non-diabetic) animal models. For instance, studies on healthy rats and rabbits have demonstrated that *Salvia officinalis* extract can exert a basal glucose-lowering effect without the need for pre-existing hyperglycemia. This distinguishes *S. officinalis* from some other medicinal plants that only show activity in diabetic models. The ability of the extract to modulate glucose levels in healthy subjects suggests a mechanism that enhances insulin sensitivity or inhibits glucose absorption, rather than merely replacing deficient insulin. Our results are consistent with those of [34], who observed similar hypoglycemic trends in healthy Wistar rats, further validating the potent metabolic regulatory role of this plant across different species.

Despite the significant findings of this investigation, certain limitations must be acknowledged. The sample size per group (n = 3) was relatively small; as this was a preliminary characterization of acute toxicity, the long-term impacts of *S. officinalis* extract on metabolic and organ functions were not assessed. Additionally, while a potent hypoglycemic effect was observed, the precise molecular mechanisms and the direct impact on serum insulin levels were not measured, as the study focused on initial safety and basal glucose modulation.

Based on these limitations, it is advisable that future research encompass broader studies utilizing diverse animal models of both sexes to investigate population variability in response to *S. officinalis*. Furthermore, comprehensive examinations into the sub-chronic and chronic impacts on hepatic and renal functions, alongside detailed histological assessments, are critical for an exhaustive safety evaluation. Finally, elucidating the specific signaling pathways and molecular mechanisms implicated in the observed glucose-lowering effects will furnish invaluable insights for potential clinical applications in diabetes management [36, 37].

5. Conclusions

This study provides preliminary experimental data that characterize the acute toxicity profile and basal glycemic effects of the methanolic extract of *Salvia officinalis* in rabbits. The findings underscore the presence of a **therapeutic window** that must be adhered to in order to maintain safety at efficacious dosages. Dose Safety and Toxicity Threshold: The No Observed Adverse Effect Level (NOAEL) for acute oral administration was successfully established at 1000 mg/kg body weight. Conversely, an observed lethal threshold was identified at approximately 2000 mg/kg, correlating with systemic toxicity, a 66.7% mortality rate, and significant body weight reduction. Basal Glucose Modulation: The extract demonstrated a significant basal blood glucose-lowering effect, even at the NOAEL dosage. Collectively, these results position *S. officinalis* as a promising candidate for further exploration in metabolic regulation, provided that dosages are optimized to avoid the identified toxicological thresholds.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

This study was not funded by any governmental or non-governmental organizations.

Acknowledgments

This work was supported by Thamar University, Republic of Yemen.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Hisham A. Al-khawlani **Conceptualization,** Hisham A. Al-khawlani; **methodology,** Hisham A. Al-khawlani.; validation, H.A.A **formal analysis**, Hisham A. Al-khawlani.; **investigation,** Aisha M. Al-Habri, Amal Y. Al-Nahary, Hadeel A. Al-mekhlafi, Hanan Y. Al-Nahary, Malak M. Al-magmhi, Maram M. Al-Mashrama, Resalh S. Jubran, Safa'a A. Amer, Shatha A. Al-Fthahy, Wala'a Y. Amran, Hisham A. Al-khawlani. Hisham A. Al-khawlani.; writing—**original draft preparation**, Hisham A. Al-khawlani.; writing—review and editing, Nabil Ali Al-Mekhlafi; visualization, Hisham A. Al-khawlani.; resources, Hisham A. Al-khawlani.

List of Abbreviations

FBG: Fasting Blood Glucose, **NOAEL:** No Observed Adverse Effect Level, **MTD:** Maximum Tolerated Dose, **AEC:** Animal Ethics Committee, **SEM:** Standard Error of the Mean, **BW:** Body Weight; **DM:** Diabetes Mellitus.

References

[1] Belhadj, S., Hentati, O., Hammami, M., Ben Hadj, A., Boudawara, T., Dammak, M., Zouari, S., El Feki, A. (2018) Metabolic impairments and tissue disorders in alloxan-induced diabetic rats are alleviated by *Salvia officinalis L.* essential oil, *Biomedicine & Pharmacotherapy* **108**: 985-995.

[2] Alarcon-Aguilar, F.J., Roman-Ramos, R., Flores-Saenz, J.L., Aguirre-Garcia, F. (2002) Investigation on the hypoglycaemic effects of extracts of four Mexican medicinal plants in normal and Alloxan-diabetic mice, *Phytotherapy Research* **16**: 383-386.

[3] Hossain, M.J., Al-Mamun, M., Islam, M.R. (2024) Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused, *Health Science Reports* **2024**: e2004.

[4] Eidi, M., Eidi, A., Zamanizadeh, H. (2005) Effect of *Salvia officinalis L.* leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats, *Journal of Ethnopharmacology* **100**: 310-313.

[5] Willcox, M.L., Elugbaju, C., Al-Anbaki, M., Lown, M., Graz, B. (2021) Effectiveness of Medicinal Plants for Glycaemic Control in Type 2 Diabetes: An Overview of Meta-Analyses of Clinical Trials, *Frontiers in Pharmacology* **12**.

[6] Anjali, Deepak, G., Pragi, Varun, K., Dimple, Monika (2024) A Review of Therapeutic Properties and Uses of *Salvia officinalis*, *Journal of Pharma Insights and Research* **2**: 146-154.

[7] Ghorbani, A., Esmaeilizadeh, M. (2017) Pharmacological properties of *Salvia officinalis* and its components, *Journal of Traditional and Complementary Medicine* **7**: 433-440.

[8] Ashkani-Esfahani, S., Noorafshan, A., Ebrahimi, A., Bahmani-Jahromi, M., Imanieh, M.-H., Ebrahimi, S., Hosseini, S., Tanideh, N. (2022) *Salvia Officinalis* Protects Pancreatic Beta-cells Against Streptozotocin-Induced Damage; A Stereological Study, *Jundishapur Journal of Natural Pharmaceutical Products* **17**: e109906.

[9] Ezema, C.A., Ezeorba, T.P.C., Aguchem, R.N., Okagu, I.U. (2022) Therapeutic benefits of *Salvia* species: A focus on cancer and viral infection, *Heliyon* **8**: e08763.

[10] Hasanein, P., Felehgari, Z., Emamjomeh, A. (2016) Preventive effects of *Salvia officinalis L.* against learning and memory deficit induced by diabetes in rats: Possible hypoglycaemic and antioxidant mechanisms, *Neuroscience Letters* **622**: 72-77.

[11] Rhaimi, S., Brikat, S., Lamtai, M., Ouhssine, M. (2023) Acute Oral Toxicity and Neurobehavioral Effects of *Salvia officinalis* Essential Oil in Female Wistar Rats, *Advances in Animal and Veterinary Sciences* **11**: 654–662.

[12] Sá, C.M., Ramos, A.A., Azevedo, M.F., Lima, C.F., Fernandes-Ferreira, M., Pereira-Wilson, C. (2009) Sage Tea Drinking Improves Lipid Profile and Antioxidant Defences in Humans, *International Journal of Molecular Sciences* **10**: 3937-3950.

[13] Christensen, K.B., Jørgensen, M., Kotowska, D., Petersen, R.K., Kristiansen, K., Christensen, L.P. (2010) Activation of the nuclear receptor PPARγ by metabolites isolated from sage (*Salvia officinalis L.*), *Journal of Ethnopharmacology* **132**: 127-133.

[14] Falya, Y., Sumiwi, S.A., Levita, J. (2013) Mini Review: Toxicity Study of Plant Extracts, *IOSR Journal of Pharmacy and Biological Sciences* **6**: 25-32.

[15] Amini, L., Mojab, F., Jahanfar, S., Sepidarkish, M., Raoofi, Z., Maleki-Hajiagha, A. (2020) Efficacy of *Salvia officinalis* extract on the prevention of insulin resistance in euglycemic patients with polycystic ovary syndrome: A double-blinded placebo-controlled clinical trial, *Complementary Therapies in Medicine* **48**: 102245.

[16] Ollanketo, M., Peltoketo, A., Hartonen, K., Hiltunen, R., Riekkola, M.-L. (2002) Extraction of sage (*Salvia officinalis L.*) by pressurized hot water and conventional methods: antioxidant activity of the extracts, *European Food Research and Technology* **215**: 158-163.

[17] Mokogwu, A.T.H., Adjekuko, C.O., Oshilonyah, U.H., Ikpefan, J.O., Eyenubo, O.B., Avwioro, O.G. (2022) Hypoglycaemic and Hypolipidemic Effects of Alcoholic Extract of Common Sage (*Salvia Officinalis*) In Streptozotocin –Induced Diabetic Rabbits, *African Journal of Biomedical Research* **25**: 243-247.

[18] Iravani, M., Mahinpour, R., Zahraei, Z., Toluei, Z., Asgari, F., Haghighipour, N. (2020) Comparison of cytotoxic and antioxidant activities and phenol content of four *Salvia L.* species from Iran, *Journal of Medicinal Plants* **19**: 59-68.

[19] Nutrizio, M., Gajdoš Kljusurić, J., Badanjak Sabolović, M., Bursać Kovačević, D., Šupljika, F., Putnik, P., Semenčić Čakić, M., Dubrović, I., Vrsaljko, D., Maltar-Strmečki, N., Režek Jambrak, A. (2020) Valorization of sage extracts (*Salvia officinalis L.*) obtained by high voltage electrical discharges: Process control and antioxidant properties, *Innovative Food Science & Emerging Technologies* **60**: 102284.

[20] Organisation for Economic Co-operation and Development OECD. (2025) *Guideline No. 497: Defined Approaches on Skin Sensitisation*. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, France, pp 20.

[21] Chow, P.K.H., Ng, R.T.H., Ogden, B.E. (2008) Using Animal Models in Biomedical Research, World Scientific Publication, *Singapore*, pp. 308.

[22] Obernier, J.A., Baldwin, R.L. (2006) Establishing an Appropriate Period of Acclimatization Following Transportation of Laboratory Animals, *Institute for Laboratory Animal Research (ILAR) Journal* **47**: 364-369.

[23] Javid, H., Moein, S., Moein, M. (2022) An investigationof the inhibitory effects of dichloromethane and methanol extracts of *Salvia macilenta, Salvia officinalis, Salvia santolinifola* and *Salvia mirzayanii* on diabetes marker enzymes, an approach for the treatment diabetes, *Clinical Phytoscience* **8**: 7.

[24] Al-Mekhlafi, N.A., Al-Badaii, F., Al-Ezzi, M.S., Al-Yamani, A., Almakse, E., Alfaqeeh, R., Al-Hatar, G., Al-Twity, M., Al-Masadi, M., Abdullah, M., Al-Qarhami, N. (2023) Phytochemical Analysis and Antibacterial Studies of Some Yemeni Medicinal Plants against Selected Common Human Pathogenic Bacteria, *Thamar University Journal of Natural & Applied Sciences* **8**: 14–18.

[25] Samer, B.S., Al-Mausmi, H., Gailan, A., Ziad, S., Saleh, M., Shalan, A., Hamdan, M., Gharab, A., Al-Ra'ai, A., Al-Ra'ai, N.A.-D., Al-Ezzi, M. (2024) Phytochemical Screening and Antibacterial Activity of Leaf Extracts from *Psiadia Punctulata*, *Thamar University Journal of Natural & Applied Sciences* **9**: 31–41.

[26] Abubakar, A.R., Haque, M. (2020) Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes, *Journal of Pharmacy and Bioallied Sciences* **12**: 1-10.

[27] Lamia, A.K.R., Al-Mashhady, A. (2016) Hypoglycemic effect of *Salvia officinalis L.* extracts on induced diabetic rabbits, *International Journal of PharmTech Research* **9**: 252-260.

[28] Kanana, F.M., Maina, C.M., Kibet, J.M., Clement, J.M. (2020) Hypoglycaemic effects of *Salvia officinalis* extracts on alloxan-induced diabetic Swiss albino mice, *Journal of Medicinal Plants Research* **14**: 518-525.

[29] Skalli, S., Hassikou, R., Arahou, M. (2019) An ethnobotanical survey of medicinal plants used for diabetes treatment in Rabat, Morocco, *Heliyon* **5**: e01421.

[30] Bouteldja, R., Doucene, R., Aggad, H., Abdi, F.Z., Belkhodja, H., Belal, A., Abdali, M., Zidane, K. (2023) Phytochemical screening, acute toxicity

and antidiabetic activity of ethanolic extract of *Salvia officinalis L*. in Wistar Rat, *Agriculturae Conspectus Scientificus* **88**: 351-357.

[31] Hinad, I., S'Hih, Y., Elhessni, A., Mesfioui, A., Ouahidi, M.l. (2022) Medicinal plants used in the traditional treatment of diabetes in Ksar Elkebir Region (North-Western Morocco), *Pan African Medical Journal* **42**: 319.

[32] Gebru, A., Sibhat, G., Hiben, M. (2014) Preliminary phytochemical screening and blood glucose lowering activity of methanol extract of *Salvia tilifolia* Vahl aerial part, *International Journal of Pharmaceutical and Biological Archive* **5**: 122-125.

[33] Ben Khedher, M.R., Hammami, M., Arch, J.R.S., Hislop, D.C., Eze, D., Wargent, E.T., Kępczyńska, M.A., Zaibi, M.S. (2018) Preventive effects of *Salvia officinalis* leaf extract on insulin resistance and inflammation in a model of high fat diet-induced obesity in mice that responds to rosiglitazone, *PeerJ* **6**: e4166.

[34] Pereira, O.R., Catarino, M.D., Afonso, A.F., Silva, A.M.S., Cardoso, S.M. (2018) *Salvia elegans, Salvia greggii* and *Salvia officinalis* Decoctions: Antioxidant Activities and Inhibition of Carbohydrate and Lipid Metabolic Enzymes, *Molecules* **23**: 3169.

[35] Oliveira, G.O., Braga, C.P., Fernandes, A.A.H. (2013) Improvement of biochemical parameters in type 1 diabetic rats after the roots aqueous extract of yacon [*Smallanthus sonchifolius* (Poepp.& Endl.)] treatment, *Food and Chemical Toxicology* **59**: 256-260.

[36] Ebru K, F., A, A., K, C. (2017) Extraction and HPLC Analysis of Sage (*Salvia officinalis*) Plant, *Natural Products Chemistry & Research* **05**: 298.

[37] Dinel, A.-L., Lucas, C., Guillemet, D., Layé, S., Pallet, V., Joffre, C. (2020) Chronic Supplementation with a Mix of *Salvia officinalis* and *Salvia lavandulaefolia* Improves Morris Water Maze Learning in Normal Adult C57Bl/6J Mice, *Nutrients* **12**: 1777.