

# Evaluation of the hypoglycemic effect of tea bags from *Hibiscus rosa-sinensis* L. On alloxan-induced diabetic mice

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## ABSTRACT

The global diabetes situation is alarming. According to the International Diabetes Federation (IDF), there were 6.7 million diabetes-related deaths in 2023. Recently, the use of teas containing medicinal extracts, including *Hibiscus* tea (*Hibiscus rosa-sinensis* L.), to help prevent, support, and manage the disease has become increasingly popular. This study aimed to investigate the hypoglycemic potential of HIBISCUS GTEA tea bags provided by the Faculty of Pharmacy, Nguyen Tat Thanh University. Diabetes was induced in Swiss mice through intraperitoneal injection of *Albino alloxan*. After 72 h, mice with blood glucose levels exceeding 239 mg/dL were selected for further experimentation. Mice with confirmed hyperglycemia were then divided into two groups: a negative control group (receiving distilled water) and a treatment group (receiving tea at a dose equivalent to 1000 mg/day in humans). Blood glucose concentration and body weight were monitored and recorded weekly. The results showed that administration of tea at a maximum dose of 5000 mg/kg body weight produced no signs of acute toxicity in the mice. Subchronic toxicity studies at two dose levels revealed no changes in general condition, hematopoietic function, liver function, or kidney structure throughout the study period. The hyperglycemia model demonstrated success rates of 16.7% at a dose of 150 mg/kg and 54.17% at 200 mg/kg. Blood glucose levels in the treatment group decreased significantly ( $p < 0.05$ ) compared to the negative control group. Overall, the findings indicate that HIBISCUS GTEA tea bags are safe and exhibit no acute or short-term repeated-dose toxicity in experimental animals. The tea preparation exhibited a significant hypoglycemic effect at a dose equivalent to 235.2 mg/kg/day in mice.

**Key words:** *Hibiscus rosa-sinensis* L., acute toxicity, short-term repeated dose toxicity, hypoglycemic potential, tea bag

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## INTRODUCTION

Currently, diabetes is becoming a serious public health problem in Vietnam and worldwide. According to estimates by the International Diabetes Federation (IDF), in 2024, there were approximately 589 million people aged 20–79 living with diabetes, accounting for 11.1% of the global population in this age group, and this number is projected to rise to 853 million by 2050<sup>1</sup>. Although many pharmacological treatments for diabetes are currently available, their long-term use may lead to undesirable effects such as excessive hypoglycemia, liver and kidney damage, or gastrointestinal disorders. Therefore, the use of products derived from medicinal plants to support blood glucose control is gaining increasing attention due to their safety, affordability, and suitability for long-term use. *Hibiscus rosa-sinensis* L. is a widely cultivated plant in many tropical countries, including Vietnam, where it is widely used in traditional medicine for its effects such as heat clearing, diuresis, blood pressure regulation, and digestive support<sup>2,3</sup>. Many recent studies have shown that *Hibiscus rosa-sinensis*

L. extracts contain important bioactive compounds, such as flavonoids and anthocyanins, which have the potential to regulate blood glucose through mechanisms including  $\alpha$ -glucosidase inhibition, antioxidant activity, enhanced insulin sensitivity, and protection of pancreatic  $\beta$ -cells<sup>4,5</sup>. Several *in vitro* and *in vivo* studies have also demonstrated the hypoglycemic effects of *Hibiscus rosa-sinensis* L. in diabetic mouse models<sup>6</sup>. However, most previous studies have focused on extracts with unstandardized compositions, which differ from the ready-to-drink tea products commonly used by consumers.

In Vietnam, hibiscus tea bags are widely consumed primarily as common beverages or traditional remedies, yet there is a lack of systematic scientific studies that clearly have evaluated their hypoglycemic effects or the safety of long-term use. Therefore, it is necessary to investigate and assess both the acute toxicity and hypoglycemic activity of hibiscus tea bags, thus providing scientific evidence to support the development of safe and effective functional food products for blood glucose management in the community.

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## MATERIALS AND METHODS

### Materials

*Hibiscus rosa-sinensis* L. tea bags (Hibiscus GTEA) were prepared under the supervision of Dr. Vo Thi Ngoc My from the Faculty of Medical Laboratory Technology, Nguyen Tat Thanh University, who was responsible for the formulation and quality control of the product. *Hibiscus rosa-sinensis* L. flowers were harvested from June to October from District 7, Ho Chi Minh City, Vietnam. The petals of *Hibiscus rosa-sinensis* L. and *Stevia rebaudiana* were extracted with water at 80°C to ensure optimal recovery of most of the flavonoids as well as steviol<sup>7,8</sup>. Each hibiscus tea bag contains 0.6 g of *Hibiscus rosa-sinensis* L. extract, 0.048 g of *Stevia rebaudiana* extract, along with dried *Hibiscus rosa-sinensis* L. The petals were ground to a semi-fine size, with 2 g used per preparation. Male Swiss albino mice (6–8 weeks old, weighing 20 ± 3 g) were obtained from the Pasteur Institute in Ho Chi Minh City, Vietnam. The animals were housed in polypropylene cages with wood-chip bedding, with a maximum of five mice per cage, under controlled environmental conditions (temperature 22 ± 2°C, relative humidity 55 ± 10%, and a 12-h light/dark cycle), and they were provided with free access to standard laboratory chow and water. Prior to experimentation, the mice were acclimatized for 72 h. Randomization into experimental groups was carried out using a computer-generated sequence, and the investigator responsible for data collection was blinded to group allocation to minimize bias. The sample size was determined based on previous studies and statistical power analysis to ensure reliability while minimizing animal use. Humane endpoints were defined according to institutional ethical standards: animals showing signs of distress, abnormal behavior, or body weight loss exceeding 20% were humanely euthanized. All procedures followed the ARRIVE guidelines and were approved by the Institutional Animal Ethics Committee of Nguyen Tat Thanh University.

Chemicals and equipments:

Alloxan monohydrate (98%, Sigma-Aldrich, USA) was used to induce diabetes in mice. Distilled water (Vietnam), distilled water for injection (B. Braun, Germany), 0.9% injectable sodium chloride solution (B. Braun), and 5% glucose injectable solution (B. Braun) were used for solution preparation and animal treatment. Blood glucose levels were measured using the On-Call Plus rapid test kit in combination with a compatible glucose meter. All other reagents and analytical instruments used in the study were of standard quality and analytical grade.

### Methods

#### Acute toxicity evaluation of *Hibiscus rosa-sinensis* L. tea bags in a mouse model

The acute oral toxicity of *Hibiscus rosa-sinensis* L. tea bags (HIBISCUS GTEA) was evaluated using a fixed-dose procedure (limit test) in accordance with Decision No. 141/QĐ-K2DT of the Ministry of Health of Vietnam. Male Swiss white mice (20 ± 3 g) were used to avoid the influence of the estrous cycle in females, which may cause physiological variability and increase error in the assessment. The extract was freshly prepared each morning in distilled water and administered orally at a volume of 0.1–0.2 mL/10 g body weight. An initial dose of 4000 mg/kg produced no signs of toxicity, leading to a confirmatory test in six mice (including the initial subject) at a dose of 5000 mg/kg. Mice were closely monitored for the first 4 hours after tea ingestion and twice daily for 7 days<sup>9</sup>. Observations included clinical signs, behavior, body weight, food intake, and excretion. Pre-defined early termination criteria and compassionate criteria: weight loss ≥ 20%, severe decrease in activity, seizures, inability to access food or water, or moribund state.

#### Short-term repeated dose toxicity assessment

This 14-day oral administration study was conducted as a preliminary range-finding (dose-setting) investigation to inform dose selection for a subsequent 28-day repeated-dose toxicity study, in accordance with OECD Test Guideline 407 and Decision No. 141/QĐ-K2DT of the Ministry of Health. Healthy adult mice (n = 8 per group; 4 males and 4 females) were randomly assigned to three groups: control, low-dose (L2000), and high-dose (L4000). The test doses were based on human equivalent doses of 2000 mg/day and 4000 mg/day for a 50-kg adult, converted to mouse doses using allometric scaling, corresponding to approximately 480 mg/kg/day (L2000) and 960 mg/kg/day (L4000), respectively. The doses were administered orally by gavage once daily at a volume of 0.1–0.2 mL per 10 g of body weight for 14 consecutive days. Throughout the study, all animals were observed daily for mortality, general health status, and clinical signs; food and water intake were periodically recorded, and fecal and urinary outputs were examined for appearance and consistency. Body weight was measured on day 0 and day 14. At the end of the treatment period, blood samples were collected for hematological and biochemical analyses to identify any potential dose-limiting effects. Subsequently, an *in vivo* evaluation of the hypoglycemic activity of the tea bags was conducted.

### *Constructing a diabetes mellitus model induced by alloxan in mice*

Based on previous studies by Lao Duc Thuan et al. in 2013<sup>10</sup> and related research, this study designed and adapted a diabetic mouse model to suit the current experimental conditions<sup>11-18</sup>. Alloxan solution at a concentration of 50 mg/mL was prepared in 0.9% sodium chloride injectable solution at 8–16°C, protected from light, and used immediately after preparation. Diabetes was induced in two groups of mice with doses of 150 mg/kg (n = 12) and 200 mg/kg (n = 24). After completing experiments with the 150 mg/kg dose group, mice were injected intraperitoneally with a single dose of the alloxan solution (50 mg/mL) following an overnight fast. The injection volume for each mouse was calculated individually based on body weight using the following formula:

$$\text{Injection volume (mL)} = \frac{\text{Dose level mouse (mg/kg)} \times \text{Mouse weight (g)}}{50000}$$

Because alloxan can induce lethal hypoglycemia by triggering massive insulin release from the pancreas, the mice were given an additional 20% (w/v) glucose solution (10 mL) 6 h after injection. They were then maintained for 24 h on 5% (w/v) glucose solution to prevent hypoglycemia<sup>12</sup>. Blood glucose levels were monitored periodically using a glucose meter, and mice with blood glucose exceeding 239 mg/dL were selected for further evaluation of the hypoglycemic effects of the tea<sup>13</sup>.

### *Evaluation of hypoglycemic effects in mice*

Diabetic mice were randomly assigned to two groups, ensuring no significant differences in baseline blood glucose levels<sup>11</sup>. The negative control group (n = 6) did not receive the HIBISCUS GTEA extract, while the experimental group (n = 6) was administered the extract via passive access in drinking water at a mouse equivalent dose (MED) of 235.2 mg/kg/day. This dose was calculated from a human equivalent dose (HED) of 20 mg/kg, corresponding to 1000 mg/day for a 50-kg individual, using standard body surface area conversion factors (Nair & Jacob et al., 2016)<sup>14</sup>. The study was conducted in accordance with Decision No. 141/QĐ-K2ĐT issued by the Ministry of Health of Vietnam.

**Preparation of the tea infusion:** The tea infusion was prepared following the method of Vo Thi Ngoc My et al. (2025)<sup>15</sup>. A 2-g tea bag (containing 0.6 g *Hibiscus rosa-sinensis* extract, 0.048 g *Stevia rebaudiana* extract, and dried hibiscus flower powder) was steeped in 50 mL of hot distilled water at 90°C for 10 min and then cooled to room temperature and filtered. The

resulting extract was concentrated to 17 mL, yielding an approximate concentration of 29.41 mg/mL. Total flavonoid content, determined via ELISA spectrophotometry, was 3.04 mg QE/g dry sample. The extract was stored at 4°C and used within 24 h. The oral administration (PO) volume (mL) was calculated as follows:

$$\text{Volume (mL)} = \frac{\text{Dose in mouse (mg/kg)} \times \text{Mouse body weight (g)}}{\text{Extract concentration (mg/mL)} \times 1000}$$

This procedure complied with the ARRIVE guidelines and relevant OECD recommendations for preclinical efficacy studies on herbal preparations.

Mice were housed individually with ad libitum access to food and water<sup>13</sup>, with the final food provision at 5 p.m. daily. Body weight, behavioral patterns, and residual food were monitored daily throughout the 16-day experimental period, which included 15 consecutive days of oral administration, with day 0 as baseline and day 15 as the predefined primary endpoint. This short-term design follows standard preclinical protocols for herbal hypoglycemic studies, allowing adequate monitoring of glycemic responses while minimizing animal stress. Tail vein blood samples were collected periodically on days 0, 3, 6, 9, 12, and 15 after overnight fasting, prior to the morning meal, for blood glucose measurement using a glucometer. All animals successfully induced with diabetes were included in the study, and no subjects were excluded. The extract was administered via controlled oral gavage (0.1–0.2 mL/10 g body weight) to ensure consistent exposure. Data were summarized as the means ± SEMs in accordance with ARRIVE guidelines.

**Mouse tail vein blood collection method:** On days 0 and 15, venous blood was collected from the tail vein of each mouse into designated sample tubes for laboratory analysis. Data from day 0 served as baseline values for comparison. Samples were analyzed using the Mindray BC-3000Plus Automated Hematology Analyzer and the Mindray BA-88A Semi-automated Chemistry Analyzer.

The resulting data were compared to evaluate the biological status of the experimental mice before and after the study. Hematological and biochemical results were interpreted according to reference standards from The Laboratory Mouse, Third Edition, 2023<sup>11</sup> and Charles River Research Model, 2012<sup>19</sup>.

### **Statistical data analysis**

The collected data were statistically analyzed using appropriate non-parametric tests, including T-test and One-Way ANOVA (or Mann-Whitney U), using SPSS

**Table 1: Hematological and biochemical parameters according to The Laboratory Mouse – Third Edition 2023 [Source: Authors]**

| Biochemical index | Glycemic index (mg/dL) | Red blood cell count (RBC) (109/ $\mu$ L) | White blood cell count (WBC) (103/ $\mu$ L) |
|-------------------|------------------------|-------------------------------------------|---------------------------------------------|
| Lower bound       | 97                     | 9.17                                      | 3.63                                        |
| Upper bound       | 239                    | 12.10                                     | 16.70                                       |

statistical software (or alternative software such as Microsoft Excel 2016, R, Python). The results are presented as the means  $\pm$  standard errors (SEMs) with  $p < 0.05$ .

## RESULTS

### Evaluation of *Hibiscus rosa-sinensis* L. tea bags in a mouse model

The preliminary test on a single mouse indicated that the animal remained alive and active after administration, with no observable signs of toxicity, suggesting that the test substance did not produce acute poisoning symptoms. The mouse's body weight showed no significant change. Although food and water were provided ad libitum, exact consumption was difficult to quantify due to normal rodent behavior. Feces and urine analysis indicated nearly complete absorption, distribution, metabolism, and excretion of the 4000 mg/kg dose after 48 h. The results of the official test conducted on six mice using the tea bag extract at 5000 mg/kg (including 01 preliminary test subjects) are presented in Figure 1.

After 7 days of testing, the body weights of all mice, both male and female, increased. Based on the preliminary data, the tea bags administered at a dose of 5000 mg/kg did not produce any noticeable adverse effects on body weight gain during the study period. This observation aligns closely with published findings by Sikarwar Mukesh et al. in 2011<sup>20</sup>, indicating a precise correlation. Analysis of feces and urine showed that absorption, distribution, metabolism, and excretion were nearly complete for the 5000 mg/kg dose after 48 h, with no significant differences compared to the preliminary test at 4000 mg/kg in a single Swiss mouse.

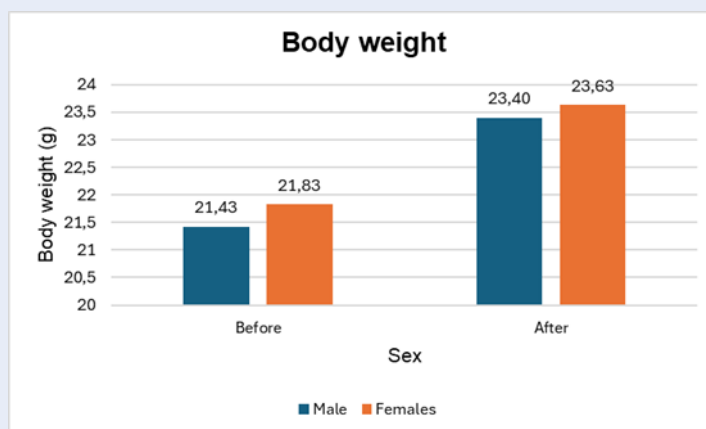
### Short-term repeated dose toxicity assessment in experimental animals

The short-term repeated dose toxicity assessment results are shown in Tables 2 and 3 and Figure 1.

Both male and female mice were used in each group ( $n = 8$  in total). As this study was conducted as a 14-day range-finding test, data from both sexes were combined for analysis to estimate general trends in tolerance and dose-related effects.

**Body weight:** Statistical analysis using one-way ANOVA revealed no significant differences in the average body weight among the three groups on both days 1 and 15. The negative control group showed a stable weight gain, reaching an average of  $29.55 \pm 0.95$  g by day 15. The experimental group L2000 showed a slight increase, with an average weight of  $28.2 \pm 0.41$  g on day 15, which was not significantly different from the control, indicating that this dose of tea extract did not significantly affect growth. The L4000 group exhibited the slowest weight gain, reaching an average of  $27.89 \pm 0.73$  g on day 15. Compared with the other two groups, the slower weight gain in the L4000 group was more pronounced, suggesting that higher doses of the tea extract may influence food absorption or energy metabolism. Nevertheless, the average body weights of all three groups remained within the normal range for developing mice. Differences in body weight among groups ranged from 2 to 3 g, which falls within the standard range of 18–40 g for mice aged 6 weeks and older (Table 3, Figure 2)<sup>13,20</sup>. The variation in growth rate between the two test doses indicates that the effect of the tea extract on body weight is dose-dependent. These results suggest that the L2000 group maintained normal physiological metabolism and growth, as reflected by stable body weight and normal hematological and biochemical indices, without signs of toxicity.

**Red blood cell count:** Data analyzed using one-way ANOVA in SPSS showed no significant differences in average red blood cell (RBC) counts among the three groups on both days 1 and 15. In the negative control group, the average RBC count increased slightly from day 1 to day 15. The L2000 group also showed a slight increase, which was not significantly different from the negative control, indicating that this dose of tea extract did not meaningfully affect hematopoiesis. The L4000 group exhibited a modest increase in RBC count over the same period, but its mean remained lower than those of the L2000 and control groups (Table 3, Figure 2). However, this difference is insufficient to draw a definitive conclusion regarding the effects of the tea extract, as red blood cell counts can naturally fluctuate over time, even in healthy mice. Overall, the RBC counts on



**Figure 1:** Body weight index of experimental mice before and after the acute toxicity test [Source: Authors']

**Table 2:** Results of body weight and blood count in the short-term repeated dose toxicity assessment Groups [Source: Authors']

|                        | Body weight (g)               |                               | Red blood cell count ( $\times 10^6$ Cells/ $\mu$ L) |                              | White blood cell count ( $\times 10^3$ Cells/ $\mu$ L) |                               |
|------------------------|-------------------------------|-------------------------------|------------------------------------------------------|------------------------------|--------------------------------------------------------|-------------------------------|
|                        | 1 day                         | 15 days                       | 1 day                                                | 15 days                      | 1 day                                                  | 15 days                       |
| Negative control group | 23.11 $\pm$ 0.25 <sup>a</sup> | 29.60 $\pm$ 0.83 <sup>a</sup> | 8.03 $\pm$ 0.08 <sup>a</sup>                         | 9.60 $\pm$ 0.19 <sup>a</sup> | 7.07 $\pm$ 0.05 <sup>a</sup>                           | 7.99 $\pm$ 0.28 <sup>b</sup>  |
| L2000                  | 22.57 $\pm$ 0.26 <sup>a</sup> | 28.44 $\pm$ 0.34 <sup>a</sup> | 8.02 $\pm$ 0.10 <sup>a</sup>                         | 9.76 $\pm$ 0.15 <sup>a</sup> | 7.03 $\pm$ 0.05 <sup>a</sup>                           | 6.89 $\pm$ 0.11 <sup>a</sup>  |
| L4000                  | 22.91 $\pm$ 0.28 <sup>a</sup> | 27.90 $\pm$ 0.63 <sup>a</sup> | 8.02 $\pm$ 0.07 <sup>a</sup>                         | 9.48 $\pm$ 0.13 <sup>a</sup> | 7.03 $\pm$ 0.04 <sup>a</sup>                           | 7.16 $\pm$ 0.26 <sup>ab</sup> |

Notes: Data are presented as means  $\pm$  SEMs (n = 8). Different superscript letters within the same row indicate significant differences among groups (p < 0.05, one-way ANOVA followed by Tukey's post hoc test), ns = not significant (p > 0.05).

**Table 3:** Results of biochemical indicators in the short-term repeated dose toxicity assessment [Source: Authors']

| Groups                 | ALT (U/L)                     |                               | AST (U/L)                     |                               | Creatinine (mg/dL)           |                              |
|------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|------------------------------|
|                        | 1 day                         | 15 days                       | 1 day                         | 15 days                       | 1 day                        | 15 days                      |
| Negative control group | 51.45 $\pm$ 3.13 <sup>a</sup> | 53.35 $\pm$ 3.10 <sup>a</sup> | 61.48 $\pm$ 1.70 <sup>a</sup> | 64.15 $\pm$ 1.66 <sup>a</sup> | 0.38 $\pm$ 0.01 <sup>a</sup> | 0.40 $\pm$ 0.01 <sup>a</sup> |
| L2000                  | 50.64 $\pm$ 2.93 <sup>a</sup> | 48.79 $\pm$ 2.84 <sup>a</sup> | 62.91 $\pm$ 2.52 <sup>a</sup> | 60.70 $\pm$ 2.53 <sup>a</sup> | 0.39 $\pm$ 0.01 <sup>a</sup> | 0.40 $\pm$ 0.01 <sup>a</sup> |
| L4000                  | 48.38 $\pm$ 3.56 <sup>a</sup> | 46.21 $\pm$ 3.61 <sup>a</sup> | 61.66 $\pm$ 2.18 <sup>a</sup> | 59.55 $\pm$ 2.30 <sup>a</sup> | 0.39 $\pm$ 0.01 <sup>a</sup> | 0.41 $\pm$ 0.01 <sup>a</sup> |

Notes: Data are presented as means  $\pm$  SEMs (n = 8). Different superscript letters within the same row indicate significant differences among groups (p < 0.05, one-way ANOVA followed by Tukey's post hoc test). Values with the same letter are not significantly different (p > 0.05).

day 1 were outside the limits reported in *The Laboratory Mouse*, but they fell within the reference ranges of  $7.68\text{--}11.19 \times 10^6$  cells/ $\mu\text{L}$  for males and  $7.75\text{--}11.25 \times 10^6$  cells/ $\mu\text{L}$  for females, as published by Charles River Laboratories (2012)<sup>20</sup>, and were consistent with data from Karyn E. O'Connell et al. (2015)<sup>21</sup> and Linden et al. (2011)<sup>22</sup>. By the end of the experimental period, the RBC counts were within the acceptable range of  $9.17\text{--}12.10 \times 10^6$  cells/ $\mu\text{L}$ , as defined in *The Laboratory Mouse* (2023) *White blood cell count*. In the negative control group, the average white blood cell (WBC) count increased from day 1 to 15, reflecting the generally good initial health status of the animals. This slight increase may be attributed to normal physiological variations or environmental factors related to housing. In the L2000 group, the average WBC count on day 15 was slightly lower than that of the negative control group. Although the decrease was small, it may suggest that the tea extract at this dose could influence physiological activity. The L4000 group showed a slight increase in average WBC count compared to both the negative control and L2000 groups on day 15 (Table 2). Nevertheless, the mean WBC count of the group remained within the reference ranges reported in *The Laboratory Mouse* of  $3.63\text{--}16.70 \times 10^3$  cells/ $\mu\text{L}$  and within the ranges of  $5.38\text{--}12.55 \times 10^3$  cells/ $\mu\text{L}$  for males and  $4.35\text{--}11.62 \times 10^3$  cells/ $\mu\text{L}$  for females, as published by Charles River Laboratories (2012)<sup>13</sup>.

**Alanine aminotransferase (ALT) and serum glutamic pyruvic transaminase (SGPT):** Data analyzed using one-way ANOVA in SPSS showed no statistically significant differences in average ALT levels among the three groups on both days 1 and 15. In the negative control group, the average SGPT increased slightly from day 1 to 15, while in the experimental groups (L2000 and L4000), ALT levels slightly decreased over the same period (Table 3). The slight increase in SGPT in the control group may be due to normal developmental changes in liver function or environmental factors, and blood collection or experimental stress could contribute to small physiological variations. At both experimental doses, the tea extract appeared to have a neutral or slightly positive effect on liver function. All recorded values remained within the reference ranges reported in *The Laboratory Mouse*: 40–86 U/L for males and 33–56 U/L for females<sup>13</sup>. These minor fluctuations in SGPT are likely attributable to normal physiological factors.

**Aspartate transaminase (AST) and glutamic-oxaloacetic transaminase (SGOT):** Data analyzed using one-way ANOVA in SPSS showed no significant differences in average AST levels among the

three groups on both days 1 and 15. The negative control group exhibited a slight increase in SGOT, while the L2000 group showed a mild decrease, and the L4000 group demonstrated a more pronounced reduction from days 1 to 15 (Table 3). Despite these changes, the AST and SGOT values remained within the reference ranges reported in *The Laboratory Mouse* (49–156 U/L for males and 44–94 U/L for females)<sup>13</sup>. The observed decrease in AST in the tea extract groups suggests a potential hepatoprotective effect, consistent with findings reported in previous studies.

**Creatinine:** Statistical analysis using one-way ANOVA showed no significant difference in mean creatinine levels among the three groups on either day 1 or 15 ( $p > 0.05$ ). Although all groups exhibited a slight increase in creatinine from day 1 to 15, this change was not statistically significant and remained within the normal physiological range for mice (0.3–0.5 mg/dL), as reported in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and by Charles River Research Models (2012)<sup>19,23</sup>. These results indicate that administration of *Hibiscus rosa-sinensis* L. tea did not adversely affect renal function under the tested conditions.

## Verification of the alloxan-induced diabetes model in mice

The results confirmed that diabetes was successfully induced, as evidenced by significantly elevated fasting blood glucose levels and consistent survival across test groups (Table 4, Figure 2).

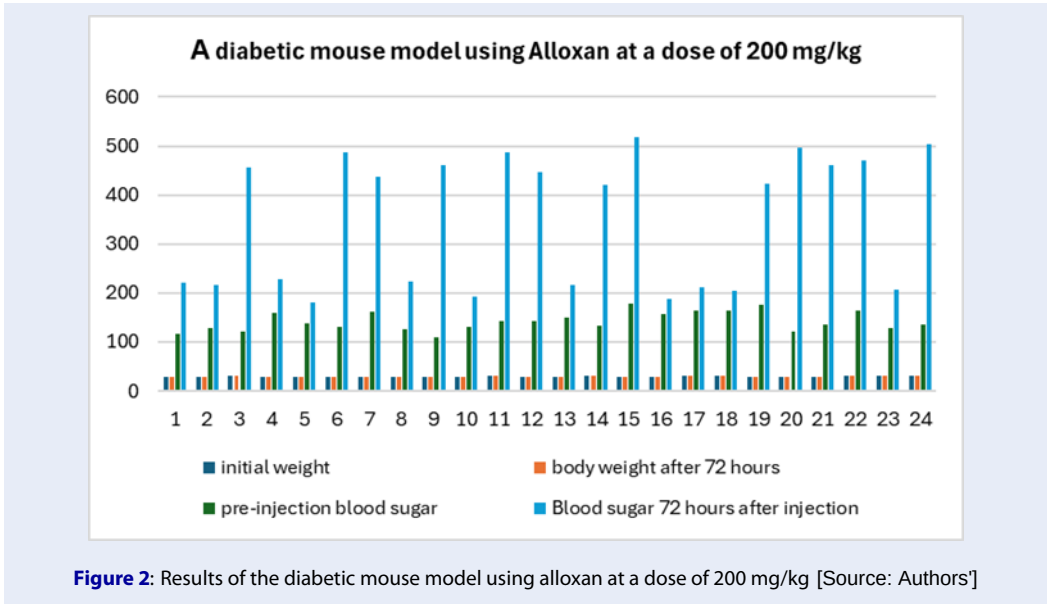
Data analyzed using a paired-sample t-test in SPSS showed significant differences in both body weight and blood glucose levels before and after alloxan injection. After administration, elevated blood glucose levels confirmed the diabetogenic effect of alloxan, particularly at the 200 mg/kg dose. One-way ANOVA revealed no difference in baseline blood glucose between the two experimental groups on day 1, but a significant difference emerged by the last day. Alloxan effectively damages pancreatic  $\beta$ -cells and induces diabetes in mice in a dose-dependent manner. At 150 mg/kg, 2 of 12 mice developed hyperglycemia, yielding a success rate of 16.7%, whereas at 200 mg/kg, 13 of 24 mice reached diabetic levels, corresponding to a 54.17% success rate. These results indicate that 200 mg/kg is a more effective dose for inducing diabetes in mice<sup>5</sup>.

## Evaluation of the hypoglycemic effect in mice

Statistical analysis showed no significant differences in mean body weight between the negative control

**Table 4:** Results of the feasibility study of the alloxan-induced diabetes model in mice [Source: Authors']

| Dosage    | Number of tests | Number of diabetic mice | Percentage of mice surviving the test (%) | Success rate (%) |
|-----------|-----------------|-------------------------|-------------------------------------------|------------------|
| 150 mg/kg | 12              | 2                       | 100                                       | 17               |
| 200 mg/kg | 24              | 13                      | 100                                       | 54.17            |



and experimental groups on days 0 and 3 ( $p > 0.05$ ). However, from day 6 to 15, mice treated with *Hibiscus rosa-sinensis* L. tea exhibited significantly higher body weights compared to the control group ( $p < 0.05$ ) (Table 6).

Values with the same letter are not significantly different ( $p > 0.05$ ).

The differences in body weight between the two groups also reflect the severity of typical diabetic symptoms. In the early stages of the experiment, both groups showed steady weight gain, with no significant differences. However, in the later stages, the experimental group continued to gain weight, whereas the control group experienced significant weight loss. In the experimental group, body weight increased gradually, approaching 30 g, and the mice remained active. These results indicate that *Hibiscus rosa-sinensis* L. tea was effective in stabilizing body weight growth in the treated mice (Table 5).

The significant increase in body weight observed in the experimental group from day 9 onward may be attributed to improved glucose utilization and energy metabolism following administration of *Hibiscus rosa-sinensis* L. tea. Flavonoids and anthocyanins in the extract have been reported to enhance insulin sensitivity, reduce hyperglycemia-induced catabolism,

and support the restoration of normal metabolic activity<sup>4,6</sup>. As a result, diabetic mice receiving the tea extract were able to maintain or gain body weight, while the negative control group continued to lose weight due to uncontrolled hyperglycemia.

The results showed that from day 3 to 15, there was a clear difference in average blood glucose levels between the two groups. The experimental findings demonstrated that HIBISCUS GTEA effectively stabilized blood glucose in diabetic mice at a dose equivalent to 1000 mg/day in humans (approximately 235.2 mg/kg body weight in mice). In contrast, the negative control group exhibited progressive hyperglycemia over time without supportive treatment. These results are consistent with previous studies by Sneha S. Pillai and S. Mini, which showed that the flavonoid-rich fraction from *Hibiscus rosa-sinensis* L. (EHRS) petals could enhance insulin secretion under high-glucose conditions and reduce inflammatory and apoptotic signaling, thus protecting  $\beta$ -cells from hyperglycemia-induced damage<sup>4</sup>. Additionally, anthocyanins in *Hibiscus rosa-sinensis* L. petals can inhibit alpha-glucosidase, slowing intestinal glucose absorption into the bloodstream<sup>5</sup>.

Thus, this initial research confirms the effectiveness of Hibiscus tea bags in decreasing and stabilizing blood

**Table 5: Results of the body weight survey on the experimental mouse model over time [Source: Authors']**

| Groups                     | Day 0                     | Day 3                     | Day 6                     | Day 9                     | Day 12                    | Day 15                    |
|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| The negative control group | 29.47 ± 0.36 <sup>a</sup> | 31.17 ± 0.41              | 32.75 ± 0.34 <sup>a</sup> | 31.17 ± 0.36 <sup>a</sup> | 29.45 ± 0.43 <sup>a</sup> | 27.82 ± 0.50 <sup>a</sup> |
| The experimental group     | 29.55 ± 0.32 <sup>a</sup> | 31.50 ± 0.34 <sup>a</sup> | 33.44 ± 0.35 <sup>a</sup> | 35.30 ± 0.35 <sup>b</sup> | 37.09 ± 0.35 <sup>b</sup> | 38.75 ± 0.41 <sup>b</sup> |

Data are presented as means ± SEMs (n = 8). Different superscript letters within the same column indicate significant differences between groups at p < 0.05 (Independent t-test).

**Table 6: Results of fasting plasma glucose test (FPG) on the experimental mouse model [Source: Authors']**

| Groups                     | Day 0                 | Day 3                 | Day 6                 | Day 9                | Day 12                | Day 15               |
|----------------------------|-----------------------|-----------------------|-----------------------|----------------------|-----------------------|----------------------|
| The negative control group | 463 ± 14 <sup>a</sup> | 450 ± 12 <sup>a</sup> | 447 ± 11 <sup>a</sup> | 517 ± 7 <sup>a</sup> | 534 ± 16 <sup>a</sup> | 529 ± 9 <sup>a</sup> |
| The experimental group     | 465 ± 11 <sup>a</sup> | 351 ± 14 <sup>b</sup> | 241 ± 9 <sup>c</sup>  | 245 ± 8 <sup>c</sup> | 229 ± 11 <sup>c</sup> | 224 ± 6 <sup>c</sup> |

Notes: Data are presented as means ± SEMs (n = 8). Different superscript letters within the same column indicate significant differences between groups (p < 0.05, Independent t-test). Values with the same letter are not significantly different (p > 0.05).

**Table 7: Blood count results at the beginning and end of the experiment in the experimental mouse model after 14 days [Source: Authors']**

| Complete blood count              | Initial white blood cell count    | Final white blood cell count | Initial red blood cell count      | Final red blood cell count |
|-----------------------------------|-----------------------------------|------------------------------|-----------------------------------|----------------------------|
| <i>Unit of measure</i>            | <i>(x10<sup>3</sup> Cells/μL)</i> |                              | <i>(x10<sup>6</sup> Cells/μL)</i> |                            |
| <i>The negative control group</i> | 7.66 ± 0.27 <sup>a</sup>          | 8.84 ± 0.19 <sup>b</sup>     | 9.37 ± 0.21 <sup>a</sup>          | 10.75 ± 0.27 <sup>a</sup>  |
| <i>The experimental group</i>     | 7.74 ± 0.33 <sup>a</sup>          | 7.35 ± 0.28 <sup>a</sup>     | 9.52 ± 0.18 <sup>a</sup>          | 9.84 ± 0.14                |

Notes: Data are presented as means ± SEMs (n = 8). Different superscript letters within the same column indicate significant differences between groups at p < 0.05 (Independent t-test). Values with the same letter are not significantly different (p > 0.05).

sugar in diabetic animals. This is the basis for further studies to continue to evaluate and compare the effects and bioavailability of this product with commercial diabetes medications such as Metformin to find an alternative to chemical drugs in the treatment of diabetes.

Statistical analysis showed that the two experimental groups had comparable mean RBC and WBC counts at baseline. However, significant differences were observed after the 15-day treatment period.

Comparison of CBC results between the two groups showed that the mean WBC count remained within the reference range of  $3.63\text{--}16.70 \times 10^3$  cells/μL, as defined by *The Laboratory Mouse*, and was consistent with the range of  $5.38\text{--}12.55 \times 10^3$  cells/μL reported by Charles River Research Models in both assessments. However, at the end of the study, the WBC count in the experimental group was lower than in the control group. This effect is likely due to the anti-inflammatory properties of the *Hibiscus sabdariffa* tea extract, which may help mitigate chronic inflammatory complications associated with diabetes mellitus. Consequently, WBC counts increased in the control group while remaining stable in the experimental

group. A significant difference was also observed in the final RBC counts, which were higher in the control group compared to the experimental group. Nevertheless, RBC counts in both groups remained within the reference range of  $9.17\text{--}12.10 \times 10^6$  cells/μL as defined by *The Laboratory Mouse*. Moreover, when compared with relevant studies, including the findings of Giorgio Silva-Santana et al. (2020) and reference values reported by Charles River Research Models, the RBC counts in both groups at the end of the study remained within the normal evaluable range.

## CONCLUSION

The research results indicate that *Hibiscus rosasinensis* L. tea bags (HIBISCUS GTEA) are safe and may support blood glucose reduction. In the short-term acute toxicity assessment, mice administered high doses ranging from 4000 to 5000 mg/kg body weight showed no signs of toxicity during 7 days of monitoring. In the 14-day repeated-dose toxicity study, mice receiving doses equivalent to 480 mg/kg/day (L2000) and 960 mg/kg/day (L4000) exhibited no abnormalities in body weight, blood count,

hibited no abnormalities in body weight, blood count, or biochemical indices. In the evaluation of hypoglycemic efficacy, diabetic mice were successfully induced with alloxan at a 54.17% success rate. When administered at a dose equivalent to 1000 mg/day in humans, *Hibiscus rosa-sinensis* L. tea bags produced a clear and stable reduction in blood glucose levels in diabetic mice, accompanied by a positive effect on body weight. These findings demonstrate that *Hibiscus rosa-sinensis* L. tea bags were non-toxic under the experimental conditions and exerted a practical hypoglycemic effect in diabetic mouse models. The study also provides a foundation for future studies, including clinical pilot trials to evaluate the hypoglycemic efficacy and safety of HIBISCUS GTEA in human participants.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHORS' CONTRIBUTIONS

The surgical and medical practices were conducted by Vo Thi Ngoc My and Nguyen Huu Khuong. The concept of the study was developed by Vo Thi Ngoc My and Le Thu Thuy. Data collection and processing were performed by Nguyen Huu Khuong and Le Thu Thuy. Analysis and interpretation of the data were undertaken by Vo Thi Ngoc My. The literature search was conducted by Le Thu Thuy, and the manuscript was written by Vo Thi Ngoc My.

## REFERENCES

- Duncan BB, Magliano DJ, Boyko EJ. IDF diabetes atlas 11th edition 2025: global prevalence and projections for 2050. *Nephrology Dialysis Transplantation*. 2025;p. gfaf177.
- Tawfeeq A, Zaini AA, Rahman M, Saleh A. Mini review: Phytochemistry and pharmacological activity of *Hibiscus rosa-sinensis* plant. *Protein: Journal of Biological Sciences*. 2023;2(4):215–223. Available from: <https://doi.org/10.61132/protein.v2i4.668>
- Roy S, Das R, Sharma C, Mohanty J. An updated review on various potential pharmacological activities of *Hibiscus rosa-sinensis* L. *Int J Biol Pharm Allied Sci*. 2023;.
- Pillai SS, Mini S. Attenuation of high glucose induced apoptotic and inflammatory signaling pathways in RIN-m5F pancreatic  $\beta$  cell lines by *Hibiscus rosa sinensis* L. petals and its phytoconstituents. *Journal of Ethnopharmacology*. 2018;227:8–17. Available from: <https://doi.org/10.1016/j.jep.2018.08.022>.
- Loganathan C, Ameen F, Sakayanathan P, Islam MA, Thayumanavan P. Exploring the interaction of phytochemicals from *Hibiscus rosa-sinensis* flowers with glucosidase and acetylcholinesterase: An integrated in vitro and in silico approach. *Computational Biology and Chemistry*. 2024;108:107996. Available from: <https://doi.org/10.1016/j.compbiolchem.2023.107996>
- Bhaskar A, Vidhya VG. Hypoglycemic and hypolipidemic activity of *Hibiscus rosa sinensis* Linn on streptozotocin-induced diabetic rats. *International Journal of Diabetes in Developing Countries*. 2012;32:214–218. Available from: <https://doi.org/10.1007/s13410-012-0096-9>.
- Tran HK, Nguyen NPN, Nguyen TTT, Nguyen KN, Do BD, Nguyen TVA, et al. Extraction of flavonoids from durian (*Durio zibethinus*) fruit rinds and evaluation of their antioxidant, antidiabetic and anticancer properties. *International Journal of Food Science and Technology*. 2024;59(3):1409–1420. Available from: <https://doi.org/10.1111/ijfs.16886>.
- Yildiz-Ozturk E, Tag O, Yesil-Celiktas O. Subcritical water extraction of steviol glycosides from *Stevia rebaudiana* leaves and characterization of the raffinate phase. *The Journal of Supercritical Fluids*. 2014;95:422–430. Available from: <https://doi.org/10.1016/j.supflu.2014.10.017>
- Ministry of Health - Department of Science, Technology and Training. Decision No. 141/QĐ-KĐTĐ. Hanoi; 2015.
- Lao DT, Nguyen TTX, Nguyen VTT, Pham HPL. Construction of diabetic mouse model and investigation of hypoglycemic effect of Hoang Lien (Copis teeta Wall) on animal model. *Ho Chi Minh City Open University Journal of Science*. 2013;8(1).
- Pethe M, Yelwatkar S, Manchalwar S, Gujar V. Evaluation of biological effects of hydroalcoholic extract of *Hibiscus rosa sinensis* flowers on alloxan induced diabetes in rats. *Drug Research*. 2017;67(08):485–492. Available from: <https://doi.org/10.1055/s-0043-109434>
- Agwaya MS, Vuzi PC, Nandutu AM. Hypoglycemic Activity of Aqueous Root Bark Extract *Zanthoxylum chalybeum* in Alloxan-Induced Diabetic Rats. *Journal of Diabetes Research*. 2016;2016(1):8727590. Available from: <https://doi.org/10.1155/2016/8727590>
- Suckow MA, Hashway S, Pritchett-Corning KR. The laboratory mouse. CRC Press; 2023. Available from: <https://doi.org/10.1201/9780429353086>
- Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharma*. 2016;7(2):27–31. Available from: <https://doi.org/10.4103/0976-0105.177703>
- Nguyen TTT, Nguyen DNT. Hypoglycemic effect of extracts from *Merremia tridentata* L. *Convolvulaceae* on alloxan-induced hyperglycemia in mice. *Journal Of Science Of Lac Hong University*. 2021;12:001–003.
- Etuk EU. Animals models for studying diabetes mellitus. *Agric Biol JN Am*. 2010;1(2):130–134. Available from: <https://doi.org/10.1111/j.1464-5491.2005.01499.x>
- Dra LA, Sellami S, Rais H, Aziz F, Aghraz A, Bekkouche K. Antidiabetic potential of *Caralluma europaea* against alloxan-induced diabetes in mice. *Saudi Journal of Biological Sciences*. 2019;26(6):1171–1178.
- Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*. 2017;53(6):365–374. Available from: <https://doi.org/10.1016/j.medic.2018.02.001>
- Laboratories CR. Charles River Research Model - Mouse Hematology & Biochemistry. North American; 2012.
- Mukesh SS, Patil MB. Antihyperlipidemic effect of ethanolic extract of *Hibiscus rosa sinensis* flowers in hyperlipidemic rats. *RGUHS J Pharm Sci*. 2011;1(2):117–122.
- O'Connell KE, Mikkola AM, Stepanek AM, Vernet A, Hall CD, Sun CC. Practical murine hematopathology: a comparative review and implications for research. *Comparative Medicine*. 2015;65(2):96–113.
- Linden M, Ward JM, Cherian S. Hematopoietic and lymphoid tissues. Academic Press; 2012.
- Albus U. Guide for the care and use of laboratory animals. 8th ed.; 2012.