# Evaluation of the Blood Glucose-Lowering Effect of the Aqueous Leaf Extract of *Quassia amara* L. (Simaroubaceae) on Alloxan-induced Diabetes in Male ICR Mice (*Mus musculus*)

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## ABSTRACT

**Background and Objective.** Diabetes, a prevalent metabolic disorder characterized by hyperglycemia primarily due to insulin action and secretion, poses significant health challenges, particularly in low to medium-income countries such as the Philippines. *Quassia amara*, a shrub indigenous to South America and present in the Philippines, holds a rich history of utilization in alternative and complementary therapies. While previous studies have demonstrated the hypoglycemic effects of *Quassia amara* stem wood, investigations into the potential impact of its leaves on blood glucose levels remain scarce. Thus, this study aimed to assess the blood glucose-lowering effects of the aqueous leaf extract of *Quassia amara* (ALQa) on ICR strain mice.

**Methods.** Diabetes was induced in thirty male ICR mice via intraperitoneal administration of alloxan monohydrate (200 mg/kg) dissolved in 0.9% Normal Saline. The mice were divided into five groups (n=6), Group I: negative control (distilled water), Group II: reference standard glibenclamide (4 mg/kg): Groups III-V: three doses of ALQa (125, 250, and 500 mg/kg) via oral gavage. A glucometer was used to monitor the fasting blood glucose levels at 0, 1-, 2-, 6-, and 24-hour post-administration.

**Results.** Administration of alloxan monohydrate increased the FBS in the treated group to diabetic levels of >200 mg/dL. The treatment of diabetic mice with ALQa extract significantly reduced fasting blood sugar (FBS) levels in a dose-dependent manner with the highest dose of ALQa (500 mg/kg) having glucose-lowering effects comparable to glibenclamide beginning with the 2-hour mark until 24-hour post-intervention. The mean FBS at 0-hour (baseline) and 1-hour post-intervention were similar for all the groups. However, there was an increase in the mean FBS of the negative control group treated with distilled water in the first hour while there was already a decrease in the FBS of those allocated to glibenclamide and the three doses of ALQa. At both the second and 6-hour mark post-intervention, the mean FBS of the mice treated with ALQa 250 mg/kg and 500 mg/kg was comparable to glibenclamide. Finally, at the 24$^{th}$ hour post-intervention, only the mice allocated to 500 mg/kg of ALQa had comparable FBS to



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glibenclamide. The degree of reduction [mean percent reduction] of the FBS from baseline to the 24th hour was 78% for glibenclamide and 69% for ALQa 500 mg/kg (p =0.816).

**Conclusions.** The aqueous extract of *Quassia amara* leaf at 250 and 500 mg/kg produced a dose-dependent significant blood glucose-lowering effect in the alloxan-induced diabetic mice model. The 500 mg dose demonstrated a statistically comparable reduction in FBS to glibenclamide from the 2-hour time point. These findings suggest the potential of ALQa as an antidiabetic agent. Thus, warranting further investigation into its therapeutic mechanisms and clinical applications.

*Keywords: Quassia amara, alloxan monohydrate, diabetes, blood glucose-lowering*

## INTRODUCTION

Diabetes is a group of metabolic diseases with high blood glucose levels (hyperglycemia) due to insulin defects. Type 1 (T1DM) involves autoimmunity attacking pancreatic beta cells, while Type 2 (T2DM) has insulin resistance and deficiency, leading to organ damage.[1]

Globally, T2DM affects about 6.28% of the population, particularly concerning low- to middle-income countries (LMIC).[2-4] In the Philippines, an LMIC, the overall diabetes prevalence in 2013 was 6.0% with significant undiagnosed cases and related deaths. Managing T2DM imposes substantial expenses on individuals in the country.[3,5]

Diabetes treatment focuses on normalizing blood glucose levels through insulin, lifestyle changes, and oral hypoglycemic agents.[6] The WHO recognizes the significance of investigating plant-derived hypoglycemic agents.[7] Several Philippine plants, including ampalaya, avocado, banaba, bankoro, and bani, have been scientifically proven to possess hypoglycemic properties.[8–13]

*Quassia amara*, also known as Amargo or bitter ash, is a shrub native to South America.[14–17] Traditionally, it has been used as a bitter tonic for various ailments like malaria, ulcers, and stomach aches.[14,18] *Quassia amara* L. has no toxicity at certain dosages and is listed as a permitted food additive in the USA.[18,19] The quassinoid compound called quassin is responsible for its bitter taste and pharmacological effects.[20–22] It has been used in Costa Rica, Panama, and Guatemala to stimulate appetite and lower blood sugar levels.[14,23] While not traditionally used in the Philippines, studies have explored its antiprotozoal, cardiovascular, and respiratory functions.[16,17]

Quassin, a pharmacologically active compound, is abundant in all parts of the *Quassia amara* L. plant.[14,20–22] However, there is limited scientific literature on the blood glucose-lowering effects of *Quassia amara* leaves, despite their traditional use for diabetes management.[14] This study aims to investigate the blood glucose-lowering effects of the aqueous leaf extract of *Quassia amara* using a mouse model with diabetes induced by alloxan.

## MATERIALS AND METHODS

The present study employed a pretest-posttest parallel group experimental design to investigate the blood glucose-lowering effects of the aqueous leaf extract of *Quassia amara* Linn. (ALQa) on alloxan-induced diabetes in 30 male ICR mice. The study was conducted at the Animal House of the Department of Pharmacology and Toxicology, University of the Philippines Manila from November 2023 to March 2024.

### Ethical Approval

The animal experiment was registered with the Research Grants Administration Office (RGAO) with Reference number RGAO-2023-0895. It was conducted in stringent adherence to the highest ethical standards and by the protocols approved by the UPM-Institutional Animal Care and Use Committee (IACUC) with protocol number 2023-009. All necessary precautions were taken to ensure the animals' welfare and to minimize any potential pain or discomfort they may experience throughout the study. Furthermore, before initiating the research, comprehensive ethical approval was sought from both the UPM Research Ethics Board with reference number UPMREB 2023-0555-EX and the Institutional Biosafety and Biosecurity Committee (IBBC) of the University of the Philippines Manila with protocol number 2023-046.

### Equipment and Reagents

The experimental setup involved the utilization of various equipment and reagents. These included the Easy Touch Glucose Cholesterol Uric acid (GCU) meter manufactured by Bioptik Technology Inc. Taiwan, Model ET-322BT which was tested and passed the standards, and 1-ml syringes procured from a commercial seller of medical devices in Manila, Philippines; USP Standard glyburide (glibenclamide USP 12601 with CAT NO 1295505), and alloxan monohydrate 99% (obtained from Sigma-Aldrich Co, Lot # BCBX0671, purchased from Chemline Scientific Corporation).[24] Dextrose powder was sourced from Mercury Drugstores, Manila. Whatman No. 1 filter paper, glass funnels, 100mL beakers, 0.9% Normal Saline, weighing balance, oven dryer (Model DGH 9030, China) and milling machine (Model; SBF 40TV 1000) were available at the UPCM-DPT Laboratory. Freeze dryer outsourced to justfruitsinc, Las Piñas Philippines, a company known for freeze drying. Oral gavage needles (Gauge 18 X 80 mm straight) were purchased from the MOTs Animal House – an accredited commercial provider of laboratory supplies. Additionally, distilled drinking water was procured from Watsons Philippines. Corn cob beddings and Altronmin pellet feeds were acquired from Dayvco Bio Philippines Corp. An iPhone 14 pro max camera was employed during the in-vivo assay for the documentation process.

## Preparation of the Aqueous Leaf Extract of *Quassia amara*

The fresh matured leaves of *Quassia amara* L. were collected during the rainy season. The harvesting process involved gathering leaves from all sides of the *Quassia amara* tree at the former NIRPROMP-IHM Agricultural Medicinal Plants Garden, University of the Philippines, Los Baños (UPLB), Laguna. The harvested leaves were authenticated by Edwino S. Fernando Ph.D. of the Institute of Biology, University of the Philippines Diliman.

The plant materials were sorted to remove contaminants, such as damaged leaves and attached animals. The leaves were washed, dried in the shade, then oven-dried at 60˚C to a water content of less than 10%, and ground into a coarse powder. A known quantity of the powdered leaves (W1 = 1000 g) was soaked in distilled water in a beaker and boiled with a hotplate, the sign of a bubble indicated boiling, and this was left for about 15 minutes. The solution was filtered using cheesecloth and Whatman filter paper No. 1. The filtrate was freeze-dried. The lyophilized extracts were weighed in an empty beaker (W0), and the weight of the beaker plus the extract (W2 – 153.4 g) was used to calculate the percentage yield of the extract (15.34%). The extracts were then preserved in the freezer (-18 degrees) for further use.

## Preparation of Stock Solution

For this study, different stock solutions were utilized for each reagent used. The general formula for calculating the quantity of the stock solution to be received by the mice was calculated as follows:

$$\text{Quantity of stock solution} = \frac{\text{dose (mg/kg)} \times \text{weight (g)}}{\text{concentration (100 mg/mL)} \times 1000}$$

The division by 1000 is to convert the weight of the mice in grams to kilograms. Each mouse did not receive the extract over 1 mL.

### *Stock Solution for ALQa*

Ten percent (10%; 100 mg/mL) of the extract was prepared with distilled water. This was prepared by dissolving 500 milligrams of the extract in 5 mL of the solvent. This preparation was used for the three doses of aqueous leaf extract of *Quassia amara* which were 125 mg/kg, 250 mg/kg, and 500 mg/kg.

### *Stock Solution for Alloxan Monohydrate*

Alloxan monohydrate was prepared freshly on the day of induction. The dose to be induced in the mice was 200 mg/kg and a stock solution of 100 mg/mL was prepared. This was done by dissolving 1000 mg (1 g) of Alloxan in 10 mL 0.9% Sodium Chloride.

### *Stock Solution for Glibenclamide*

The glibenclamide dose was 4 mg/kg. It was administered orally through gavage. The stock solution prepared for glibenclamide was 0.4 mg/mL. This was done by dissolving 4 mg of glibenclamide powder in 10 mL of 5% DMSO solution.

## Experimental Animals

The study was conducted on 30 healthy male ICR mice of about 12 to 14 weeks, with an average weight ranging from 20 to 35 grams. Male ICR mice were selected based on their smaller size, ease of management, cost-effectiveness, maintenance, physiological similarities to humans, and availability. Additionally, the choice of male mice was preferred to avoid the potential hormonal influence which is associated with female mice. These mice were housed at the Animal House of the Department of Pharmacology and Toxicology, University of the Philippines Manila. Each mouse was housed in a polycarbonate cage with two mice accommodated in each cage. The environmental conditions within the facility were maintained at a temperature of 22 degrees Celsius (± 3 degrees Celsius), humidity ranging from 50% to 60%, and a natural light cycle of 12 hours of light followed by 12 hours of darkness. The mice were provided with a standard feed pellet (Standard Altromin purchased from Davyco Bio Philippines) containing 19.20% crude protein, 4.10% crude fat, 6.10% crude fiber, and 6.90% crude ash as well as access to Absolute distilled water ad libitum. Bedding material (Corncob bedding from Davyco Bio.) was used, and the bedding was replaced every three days. The cages were cleaned weekly using liquid detergent and 70% ethyl alcohol (EtOH).

## Induction of Experimental Diabetes Mellitus

Before experimentation, the mice were fasted for 14 hours (with water provided ad libitum) and their weights were recorded. Fasting was necessary to ensure low blood glucose levels, which enhances alloxan uptake by islet beta cells, improving its diabetogenicity. Freshly prepared alloxan monohydrate (200 mg/kg in 0.9% saline) was administered intraperitoneally to induce hyperglycemia.[25] Fasting blood glucose (FBG) levels were measured using an Easy Touch Glucose Cholesterol Uric acid (GCU) meter to establish a baseline.

Alloxan, also known as mesozalylurea, mesoxalylcarbamide, 2,4,5,6 tetraoxohexahydropyrimidine, or pyrimidinetetrone, is a uric acid derivative that exhibits high instability in water at neutral pH but remains relatively stable at pH 3.[26] When administered, alloxan and its reduction product, dialuric acid, initiate a redox cycle that generates superoxide radicals. These radicals then undergo dismutation to form hydrogen peroxide, leading to a significant increase in cytosolic calcium concentration and rapid destruction of pancreatic β-cells.[11,27,28] After administration of Alloxan, the mice were given access to food and water, along with a 5% dextrose solution to mitigate the early hypoglycemic phase. Mice were closely observed, and additional doses of alloxan monohydrate were administered at 48-hour intervals.

It took about one week for all mice to develop diabetes, defined as blood glucose levels exceeding 200 mg/dL.

To confirm diabetes, an Oral Glucose Tolerance Test (OGTT) was conducted. Mice were fasted overnight, given a glucose solution (2 g/kg), and blood glucose levels were monitored.[29] Mice with blood glucose levels above 200 mg/dL were confirmed as diabetic and used for subsequent experiments. Randomization of the mice occurred after diabetes was confirmed.

### Experimental Procedure

The blood glucose-lowering effect of the aqueous leaf extract of Quassia *amara* (ALQa) was evaluated by monitoring blood glucose levels in diabetic mice. Thirty male diabetic mice were randomly assigned to five groups (I-V), each consisting of six animals.

The mice were fasted for 14 hours, after which baseline blood glucose levels were measured. Group I (negative control) received 10 mL/kg distilled water, Group II (positive control) was administered 4 mg/kg glibenclamide, while Groups III, IV, and V were treated with 125 mg/kg (low dose), 250 mg/kg (mid dose), and 500 mg/kg (high dose) of ALQa, respectively. All treatments were administered via oral gavage.

Blood samples were obtained from the tail tips of the mice, which were snipped with sterile scissors after disinfecting with 70% ethanol. Gentle milking of the tail facilitated blood collection into a soft-style glucose strip, and glucose levels were measured in mg/dL using the Easy Touch Glucose Cholesterol Uric acid (GCU) meter glucometer.

Baseline glucose levels were determined before treatment for all groups. Post-treatment, blood glucose levels were measured at 0 (immediately after gavage), 1, 2, 6, and 24 hours. The results were recorded at each time point. Throughout the experiment, the mice had free access to water.

### Physical and Behavioral Changes in Mice

During the experiment, various physiological and behavioral characteristics of the mice were recorded. Weight was measured before and after the induction with alloxan monohydrate. Additional weight measurements were taken at 0 hours and 24 hours post-intervention. The amount of water consumed by the mice was monitored by measuring the amount of water taken by the mice during the husbandry. This was done for the period before and after the induction of alloxan monohydrate. Feeding behavior and frequency of urination were also observed.

### Statistical Analysis

The statistical analyses were conducted using SPSS software, version 22. Descriptive statistics are presented as means and standard deviations (SD). To assess overall differences among the treatment groups, a one-way analysis of variance (ANOVA) was performed. Post hoc comparisons of specific group means were conducted using the Tukey Honestly Significant Difference (HSD). Additionally, a repeated measures regression analysis was utilized to examine changes across different time points, starting from baseline (time point 0). Statistical significance was determined at an alpha level of $p \leq 0.05$.

**Table 1.** Physical and Chemical Properties of ALQa

| Parameter | Results |
|---|---|
| ***Yield of extract*** | 15.34% |
| ***Appearance*** | Coarse light-yellow mass |
| ***Consistency*** | Solid powdered form |
| ***Odor*** | Tea-like |
| ***Color*** | Golden |
| ***Solubility in water*** | Soluble |
| ***Normal saline solution*** | Soluble |
| ***pH*** | 5.99 ± 0.25 |

## RESULTS

### Physical and Chemical Properties of the ALQa

Table 1 shows the physical and chemical properties of the aqueous leaf extract of *Quassia amara*. This included the extract yield, appearance, consistency, odor, color, solubility in water and normal saline, and pH.

### Effect of Alloxan on FBS Levels of Male ICR Mice

The mean fasting blood sugar (FBS) levels for different groups of subjects before and after induction with alloxan monohydrate. Each group was treated with alloxan monohydrate at 200 mg/kg. Alloxan monohydrate raised the baseline FBS to 543.16 ± 63.05 mg/dl post-induction. This fell within the acceptable criteria for the mice to be considered diabetic.

### Oral Glucose Tolerance Test

The mice were randomly grouped into five consisting of six mice each. All groups exhibited a significant rise in blood glucose levels 30 minutes after glucose administration, peaking at this point. Following this peak, blood glucose levels decrease at 60 and 120 minutes, yet remain above the initial fasting levels. Group V demonstrates the highest peak at 30 minutes but is substantially reduced by 120 minutes (Table 2). Overall, the response to glucose intake is characterized by an initial sharp increase in FBS levels followed by a gradual decline, with some variability in the magnitude of these changes across the different groups.[29,30] Blood glucose at 120 mins greater than 200 mg/dl confirmed the mice as diabetic.

### Weight and Water Intake of the Alloxan-induced ICR Mice

Figure 1 shows the changes observed in the mice during the experiment, the weight before and after induction, then 0 hours and 24 hours after intervention.

**Table 2.** OGTT Duration

| Groups | 0 min mg/dl (± SD) | 30 mins mg/dl (± SD) | 60 mins mg/dl (± SD) | 120 mins mg/dl (± SD) |
|---|---|---|---|---|
| *I* | 206.67 ± 17.51 | 328.67 ± 46.16 | 288.83 ± 37.26 | 230.5 ± 30.67 |
| *II* | 216.33 ± 22.58 | 450.00 ± 89.90 | 355.33 ± 58.61 | 241.17 ± 32.46 |
| *III* | 204.00 ± 10.60 | 495.50 ± 103.80 | 416.83 ± 93.54 | 232.17 ± 16.90 |
| *IV* | 197.00 ± 16.06 | 484.33 ± 90.04 | 351.33 ± 70.80 | 216.50 ± 22.95 |
| *V* | 195.67 ± 23.65 | 512.50 ± 61.21 | 328.17 ± 45.70 | 14.91 |

Before induction, all groups started with similar body weights, around 25-30 grams, with some variability as indicated by the error bars (standard deviation). After induction, there was a decrease in body weight across all groups, but the differences were minor. 0-hour post-treatment, the body weights remain consistent with the "after induction" time point. At 24 hours post-intervention, all groups slightly increased body weight but generally remained within the range of 25-30 grams.

Figure 2 shows the changes observed in the mice during the increase of the weekly water intake by the mice the week before the induction and after the induction.

Before induction, all groups started with similar volumes of water intake, around 100-120 mL, with some variability as indicated by the error bars (standard deviation). After induction, there was a noticeable increase in the volume of water intake across all groups reaching approximately 220 mL.

## Effect of ALQa on alloxan-induced diabetic in male ICR mice

The effect of the ALQa on alloxan-induced diabetic male ICR mice is presented in Tables 3 and 4, and Figure 3.

Table 3 above presents the mean FBS of the different treatment groups at different time points within 24 hours.

The one-way ANOVA results indicated no significant differences among the treatment groups at the 0-hour and 1-hour intervals; significant differences were observed from the 2-hour to the 24-hour intervals. Post hoc analysis using Tukey's test revealed statistically significant differences in mean FBS levels between mice treated with distilled water ($p < 0.0001$) and ALQa 125 mg/kg ($p < 0.05$) compared to those treated with glibenclamide. The FBS of the 250 ($p = 0.636$) and 500 mg/kg ($p = 0.999$) treatment groups showed no significant difference ($p > 0.05$) when compared to glibenclamide at the 2-hour mark. At the 6-hour post-intervention mark, mice treated with distilled water ($p < 0.0001$) and ALQa 125 mg/kg ($p < 0.0001$) exhibited statistically significant differences in FBS levels compared to

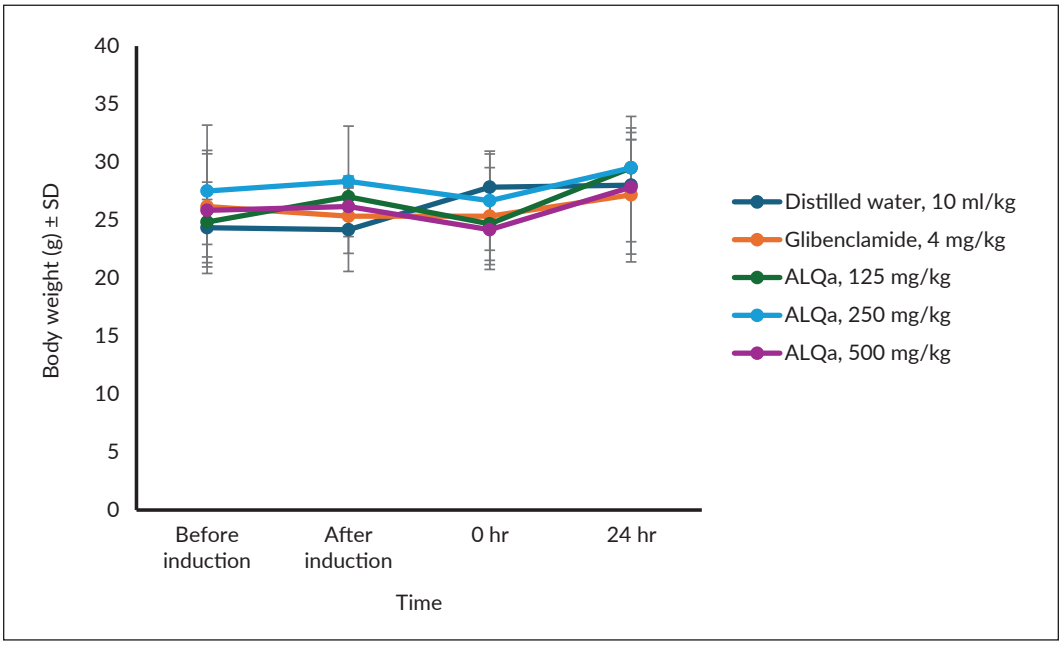


**Figure 1.** Change in body weight of ICR mice.

*ALQa = aqueous leaf extract of Quassia amara*

**Table 3.** Mean Fasting Blood Sugar (FBS) Levels (mg/dL) ± SD across Different Time Points in Alloxan-induced Diabetic Mice following various Treatments

| Groups | Treatment (n = 6) | Mean FBS (mg/dL) ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Post-alloxan | 0 hr | 1 hr | 2 hr | 6 hr | 24 hr |
| *I* | Distilled water, 10 ml/kg | 442.67 ± 188.73 | 450.50 ± 127.96 | 492.00 ± 110.16 | 489.83 ± 106.19**** | 476.50 ± 109.00**** | 470.33 ± 103.87**** |
| *II* | Glibenclamide, 4 mg/kg | 543.17 ± 63.05 | 552.00 ± 66.83 | 439.00 ± 95.91 | 356.00 ± 131.86 | 219.83 ± 91.11 | 123.33 ± 49.70 |
| *III* | ALQa, 125 mg/kg | 425.50 ± 163.59 | 453.33 ± 92.88 | 430.33 ± 96.96 | 422.00 ± 128.68* | 381.33 ± 100.07**** | 346.00 ± 118.30**** |
| *IV* | ALQa, 250 mg/kg | 419.67 ± 63.95 | 422.00 ± 100.91 | 394.50 ± 100.14 | 315.67 ± 81.26 | 221.50 ± 72.29* | 182.00 ± 61.51* |
| *V* | ALQa, 500 mg/kg | 509.67 ± 62.78 | 458.67 ± 20.20 | 404.50 ± 29.16 | 282.67 ± 70.46 | 194.67 ± 72.39 | 143.00 ± 66.64 |

**p <0.05, **p <0.01 ***p <0.001, ****p <0.0001, when compared with glibenclamide treated group*

**Table 4.** The Percentage Reduction in the FBS Level of Alloxan-induced Hyperglycemic ICR Mice Post-treatment with ALQa

| Treatment | Percentage reduction in FBS mg/dl (%) | | | |
|---|---|---|---|---|
| | 1 hr | 2 hr | 6 hr | 24 hr |
| ***Negative control*** | -12.47 ± 7.12 | -11.60 ± 5.41*** | -8.11 ± 4.69*** | -7.01 ± 4.91*** |
| ***Glibenclamide, 5 mg/kg*** | 20.43 ± 5.72 | 36.44 ± 7.78 | 60.69 ± 5.67 | 77.76 ± 3.17 |
| ***ALQa, 125 mg/kg*** | 5.26 ± 2.02 | 8.13 ± 5.80* | 15.96 ± 6.18*** | 24.47 ± 7.67*** |
| ***ALQa, 250 mg/kg*** | 6.90 ± 1.68 | 24.84 ± 3.35 | 46.77 ± 5.50 | 54.94 ± 6.51* |
| ***ALQa, 500 mg/kg*** | 11.56 ± 3.53 | 38.26 ± 6.16 | 57.65 ± 6.23 | 68.91 ± 5.74 |

**p <0.05, **p <0.01, ***p <0.0001 when compared to glibenclamide*

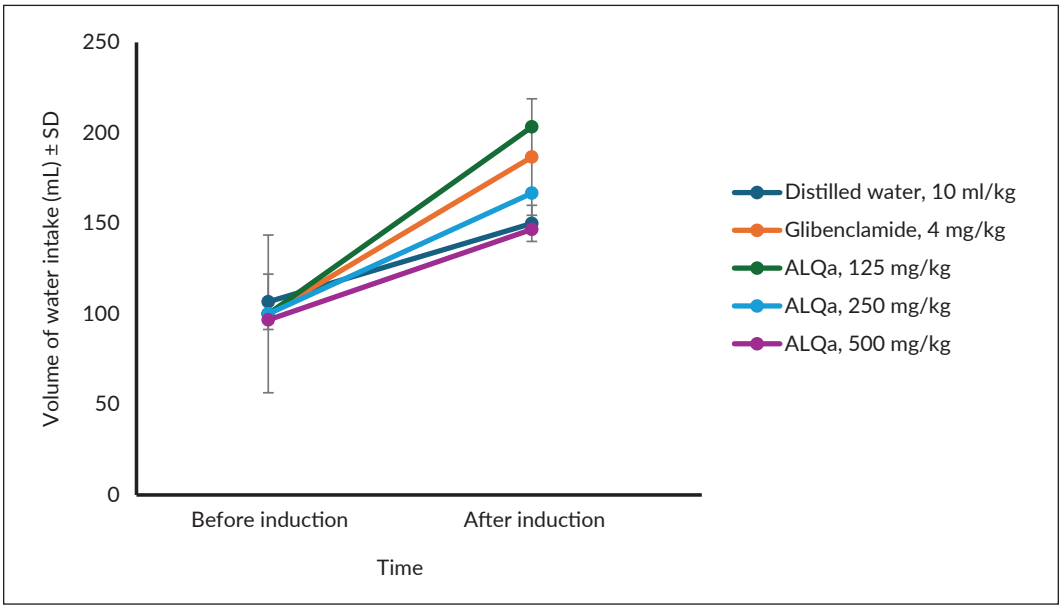


**Figure 2.** Change in the weekly water intake of ICR mice.

*ALQa = aqueous leaf extract of Quassia amara*

the glibenclamide 4 mg/kg group but there was no statistical difference (p >0.05) between the 250 (p = 0.434) and 500 mg/kg (p = 0.995) treatment groups. There were statistically significant differences noted at the 24-hour mark for the distilled water (p <0.0001), ALQa 125 mg/kg (p <0.0001), and ALQa 250 mg/kg (p <0.05) groups compared to the glibenclamide group. At 24 hours, only the ALQa 500 mg/kg (p = 0.816) dose was comparable to glibenclamide.

The group treated with glibenclamide exhibited the most reduction in FBS levels, decreasing from 543.17 ± 63.05 mg/dL post-alloxan induction to 123.33 ± 49.70 mg/dL at the 24-hour mark. This was followed by the group treated with ALQa at a dose of 500 mg/kg, which reduced FBS levels from 509.67 ± 62.78 mg/dL to 143.00 ± 66.64 mg/dL at 24 hours post-intervention, then the group treated with ALQa at a dose of 250 mg/kg had a reduction in FBS levels from

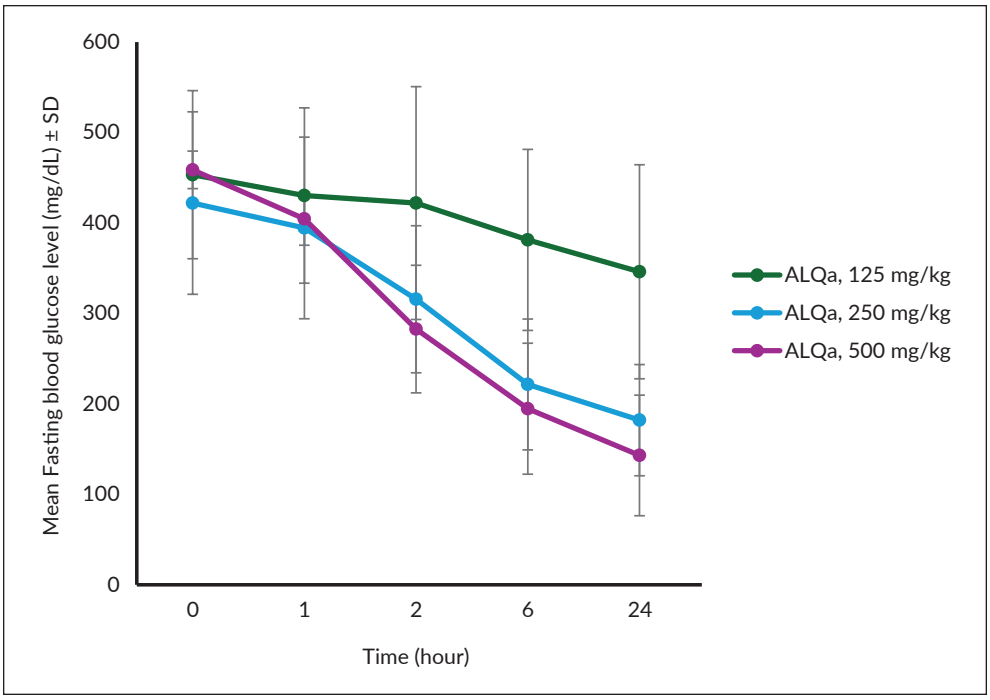


**Figure 3.** Effects of three doses of ALQa extract on the FBS of the mice.

*ALQa = aqueous leaf extract of Quassia amara*

419.67 ± 63.95 mg/dL to 182.00 ± 61.50 mg/dL over the same period. The ALQa-treated group with the highest decrease in FBS was the 500 mg/kg treatment group.

Figure 3 illustrates the dose-response curve for ALQa's effect on mean FBS levels over 24 hours. There was a decrease in the mean FBS level with increasing dose of the extract.

The serial measures comparisons indicated that each pairwise difference was significant at ($p < 0.05$). There was a significant decrease in the mean FBS over the different time points.

The ALQa when compared to glibenclamide produced a significant ($p < 0.05$) dose-dependent increase in the percentage reduction in FBS of the treated groups (Table 4). At one, two, and six hours post-treatment, there was no significant ($p > 0.05$) difference in the percentage reduction in the FBS of ALQa extract (250 and 500 mg/kg) treated groups when compared with the glibenclamide treated group. At 24 hours post-treatment, the percentage reduction in the FBS of distilled water, ALQa extract (125, 250 and 500 mg/kg), and glibenclamide-treated groups were -7.01%, 24.47%, 54.94%, 68.91%, and 77.76%, respectively.

## DISCUSSION

Type 2 Diabetes Mellitus treatment typically involves the use of established antidiabetic agents and if necessary, insulin, particularly when oral antidiabetic agents alone are insufficient in managing the hyperglycemia. There is a growing interest in utilizing medicinal plant products for managing diabetes mellitus, supported by numerous scientific studies exploring their antidiabetic properties. This study focused on assessing the potential of aqueous leaf extract of Quassia amara (ALQa) in reducing blood glucose levels in alloxan-induced diabetic mice.

The aqueous extraction method was selected for this study based on ethnopharmacological evidence supporting the traditional use of boiled *Quassia amara* leaves for diabetes management. Additionally, the boiling process is environmentally sustainable and safe in vivo studies.[14,17,31–33] The phytochemical screening of the ALQa extract showed the presence of flavonoids, steroids, alkaloids, tannins, and anthraquinones which have been reported to have antidiabetic activities.[34,35] Specifically, the steroid, quassinoid in the leaf of *Quassia amara* has demonstrated antidiabetic properties.[36,37]

Alloxan monohydrate (a pyrimidine derivative) used to induce diabetes in the mice in this study, increased the fasting blood glucose level by as much as 543.17 ± 63.05 mg/dl after seven days of alloxanization. These FBS levels of the mice post-alloxan administration were significantly higher than the values recorded by the previous studies.[38,39] The differences could be linked to the strain of the mice used and the protocol adopted in the alloxan administration.[38,39] In this study, ICR mice were used, whereas previous studies used Swiss mice.[40] A study by Tanquilut et al., utilized the ICR mice and their results gave a comparable FBS post-alloxan administration to this study.[25] Although the data was randomized, there was still a notable difference in values of the blood glucose level in the different groups post-alloxan induction.

In the induction of diabetes using alloxan, weight gain, polydipsia, polyphagia, and polyuria have been documented.[25,41] In this study, the induction of diabetes with alloxan resulted in pronounced diabetic characteristics in mice. Following induction of diabetes, the mice exhibited a marked increase in water intake, as shown in Figure 2. This polydipsic response is consistent with hyperglycemic states, where elevated blood glucose levels lead to osmotic diuresis and subsequent compensatory increase in fluid consumption.[42,43]

The mice were provided with a standard feed pellet (Standard Altromin) containing 19.20% crude protein, 4.10% crude fat, 6.10% crude fiber, and 6.90% crude ash. There was a marked increase in the animals' feeding behavior, with mice consuming more Altromin feed compared to the amounts consumed before the onset of the experiment. The diabetic mice also exhibited a notable increase in water intake, consistent with polydipsia. Urine production was similarly elevated, as evidenced by more pronounced urine presence in the bedding, indicating polyuria. These observations confirm the characteristic diabetic responses following alloxan administration. There were also no noticed signs of liver or kidney toxicities after the induction with the alloxan.

The doses of ALQa used in this study were almost similar to those utilized in previous studies.[36,44] At doses of 250 and 500 mg/kg, ALQa exhibited a blood glucose-lowering effect comparable to that of glibenclamide (4 mg/kg) in alloxan-induced diabetic mice. The highest antidiabetic activity was observed at a dose of 500 mg/kg ALQa (reduction of mean FBS by 68.91%) at 24 hours post-treatment. Comparing different doses of ALQa, there was a significant ($p < 0.05$) decrease in the mean FBS levels at doses of 250 and 500 mg/kg throughout the observation period compared to 125 mg/kg. ALQa (500 mg/kg) at 2- and 6-hours post-treatment exhibited a more rapid rate reduction in mean FBS values comparable to that of glibenclamide.

The dose-dependent increase in the percentage reduction of FBS could be linked to the higher concentration of the bioactive principle responsible for the antihyperglycemic activity at higher doses.[45] The demonstrated blood glucose-lowering effect of ALQa supports the results from other studies on the antidiabetic potential of various parts like the wood bark of *Quassia amara* L.[36,37,44] Previous studies have also reported the antihyperglycemic activity of the aqueous extract of *Quassia amara* L. wood powder at a dose of 200 mg/kg. The extract reduced the FBS of alloxan-induced hyperglycemic rats to near normoglycemic levels prior to alloxan administration.[36]

This ALQa demonstrated a reduction in fasting blood sugar levels similar to that observed with glibenclamide, which suggests that they may have same mechanism of action. Glibenclamide operates by binding to and inhibiting ATP-sensitive potassium channels on pancreatic beta cells. This action leads to a decrease in potassium efflux, depolarization of the beta-cell membrane, opening of calcium channels, influx of calcium, and subsequently, stimulation of insulin release from pancreatic beta-cells.[46]

Glibenclamide exhibits a dose-dependent effect, with an onset of action between 15 minutes and 60 minutes.[47–49] Pharmacokinetic data show that glibenclamide has a half-life of 10 hours, allowing its therapeutic effect to last up to 24 hours.[46,47] In this study, glibenclamide dosing was determined using the Animal Equivalent Dose (AED) based on the maximum recommended human dose of 20 mg/day.[50] The plant extract demonstrated a comparable hypoglycemic effect, especially in mice treated with 250 mg/kg and 500 mg/kg doses. Both doses began to reduce blood glucose levels within one hour, with the 250 mg/kg group showing a decrease from 419 mg/dL to 394.5 mg/dL, and the 500 mg/kg group reducing from 509.67 mg/dL to 404.5 mg/dL. This hypoglycemic effect was sustained over a 24-hour period, with final glucose levels of 182 mg/dL and 143 mg/dL for the 250 mg/kg and 500 mg/kg groups, respectively. These findings suggest that the plant extract provides a prolonged hypoglycemic effect that is comparable to that of glibenclamide and may have same mechanism of action. Quassinoids, specifically Quassin and neoquassin, which are prominent constituents of *Quassia amara* L., have been reported to act as insulin secretagogues.[44,51]

Another possible mechanism of the antihyperglycemic activity of ALQa extract is the inhibition of dipeptidyl peptidase-IV (DPP-IV) enzyme which is a peptidase found on the cell membrane that facilitates the stimulation of intracellular signal transduction pathways and the modulation of cell-enzyme interactions.[37] DPP-IV inhibitors have been reported to decrease blood glucose fluctuations and enhance glycemic control in type 2 diabetes mellitus (T2DM) patients.[52] It has been reported that Quassinoids, Vitexin, Quasimmarin, Simalikalactone D, Brucein D, and Quassinol isolated from *Quassia amara* have DPP-IV inhibitory activity in an in-silico study.[37]

It has also been proposed that the mechanism of the antihyperglycemic activity of ALQa may involve extra-pancreatic effect.[44] This assumption was based on the fact that the basal plasma insulin levels were not elevated after the administration of *Quassia amara* extract to experimental diabetic rats.[44] The possible extra-pancreatic effects are increased hepatic and skeletal muscle uptake of glucose and reduced insulin resistance.[53] These proposed mechanisms are not yet proven since mechanism of action studies were not directly performed in this studies.

The study findings were able to show the acute blood glucose-lowering effect of the aqueous leaf extract of *Quassia amara* L. (ALQa). The results suggest that the aqueous leaf extracts of *Quassia amara* L. exhibit a dose and time dependent antidiabetic effect in alloxan-induced diabetic mice.

## CONCLUSIONS

This study investigated the antidiabetic potential of the aqueous leaf extract of *Quassia amara* (ALQa) in alloxan-induced diabetic mice, aiming to validate

traditional use and explore its blood glucose-lowering effects. Ethnopharmacological evidence motivated the choice of aqueous extraction, and phytochemical screening confirmed the presence of bioactive compounds like flavonoids, steroids, and quassinoids, which are associated with antidiabetic activity. After alloxan induction, mice exhibited typical diabetic symptoms, including hyperglycemia, polydipsia, polyphagia, and polyuria, validating the diabetic model.

The study found that ALQa, particularly at doses of 250 mg/kg and 500 mg/kg, significantly reduced fasting blood glucose levels, showing effects comparable to glibenclamide, a standard hypoglycemic agent. Notably, ALQa's effect was sustained for up to 24 hours post-treatment. Potential mechanisms for ALQa's action include insulin secretion stimulation, DPP-IV inhibition, and extra-pancreatic effects, suggesting its multifaceted role in glucose regulation. This research supports the antidiabetic efficacy of *Quassia amara* leaf extract, highlighting its potential as a complementary approach to T2DM management.

### Limitations and Recommendations

The study focused on male ICR mice, which may limit the direct applicability of its findings to humans due to species-specific differences in physiology, metabolism, and drug response. The use of crude extract does not identify the active plant principles present in *Quassia amara,* hence, the exact mechanism of action of the active plant principle is unknown. The study's short-term duration assessed the acute effects of the extract, potentially overlooking long-term implications such as toxicity or the development of tolerance. Taking glucometer measurements reflects capillary whole blood glucose which may differ slightly from venous plasma glucose. The study did not measure the effect of *Quassia amara* on the insulin levels of the mice.

The study recommends using other strains of mice or other animals with similar pancreatic structures as humans to evaluate the antidiabetic effect of ALQa. Further studies are required to isolate and characterize the active plant principle(s) and elucidate its mechanism(s) of action and possible synergistic activity of blood glucose-lowering activity. This study focused on the acute blood glucose-lowering potential of the aqueous leaf extract of *Quassia amara,* further studies on the chronic effect on long-term use, and check for possible effects on chronic exposure since diabetes marked by hyperglycemia is a lifelong disease. Other studies can measure venous blood glucose levels, not just capillary whole blood glucose. Future studies should investigate the effects of *Quassia amara* plant extract on insulin levels to determine whether *Quassia amara* leaf extract exhibits insulin-enhancing or insulin-lowering activity.

### Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

### Author Disclosure

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