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Evaluation of the Anti-Diabetic and Tissue Protective Effects of *Aegle Marmelose* Leaf Extract in Alloxan-Induced Diabetic Mice

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KEYWORDS

Aegle marmelos, Hypoglycemic activity, Alloxan-induced diabetes, α-Amylase inhibition, Blood glucose reduction, Pancreatic protection

ABSTRACT:

This study investigates the hypoglycemic effects of aqueous extracts of *Aegle marmelos* in alloxan-induced diabetic mice. Diabetes was induced using a single intraperitoneal injection of alloxan monohydrate (180 mg/kg body weight). The aqueous extract of *Aegle marmelos* was administered orally at a dose of 500 mg/kg body weight for 14 days, resulting in a significant reduction in blood glucose levels. Phytochemical screening confirmed the presence of bioactive compounds such as tannins, flavonoids, phenols, alkaloids, and saponins, which contribute to the plant's medicinal properties. The extract exhibited strong in vitro α-amylase and α-glucosidase inhibitory activity, with IC_{50} values of 127.12 µg/mL and 147.23 µg/mL, respectively, demonstrating potential for diabetes management. Antioxidant activity assessed through the DPPH assay revealed a dose-dependent free radical scavenging effect comparable to standard antioxidants. Total phenolic and flavonoid content analysis highlighted the extract's rich bioactive composition. Acute toxicity studies indicated a high safety margin, with no observed adverse effects at doses up to 2000 mg/kg body weight. Histopathological analysis of pancreatic and liver tissues supported the extract's protective effects against alloxan-induced damage. Blood glucose monitoring confirmed a significant reduction in diabetic mice, suggesting *Aegle marmelos's* insulin-mimetic or β-cell regenerative properties. Despite its potent hypoglycemic effects, the extract prevented diabetes-associated weight loss, necessitating further studies to explore its long-term efficacy and potential integration into antidiabetic therapy. These findings support the traditional use of *Aegle marmelos* in diabetes management and highlight its therapeutic potential.

1. Introduction

Diabetes mellitus is a major global health challenge, affecting millions of people worldwide. According to the International Diabetes Federation (IDF), approximately 537 million adults were living with diabetes in 2021, a number expected to rise to 783 million by 2045. This chronic metabolic disorder, characterized by elevated blood glucose levels, results from either inadequate insulin production (Type 1 diabetes) or insulin resistance (Type 2 diabetes). The increasing prevalence of diabetes is driven by factors such as urbanization, sedentary lifestyles, unhealthy diets, obesity, and aging populations [1]. The burden of diabetes extends beyond its rising prevalence. It is a leading cause of morbidity and mortality, contributing to cardiovascular diseases, kidney failure, neuropathy, retinopathy, and lower-limb amputations. In 2021 alone, diabetes was responsible for

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6.7 million deaths globally [2]. The disease also imposes a significant economic burden on healthcare systems, with global diabetes-related health expenditures exceeding $966 billion annually. The cost is especially high in low- and middle-income countries, where access to effective treatment and management is often limited. Despite advancements in treatment, diabetes remains a progressive disease with no permanent cure. Preventive strategies such as lifestyle modifications, early screening, and public health interventions are crucial to mitigating its impact. Research into alternative therapies, including plant-based antidiabetic formulations, nanotechnology-driven drug delivery, and personalized medicine, is gaining momentum [3]. Addressing diabetes as a global burden requires a multifaceted approach involving governments, healthcare professionals, researchers, and the public. Strengthening healthcare infrastructure, promoting health education, and ensuring affordable access to diabetes management are essential to reducing its devastating impact. Without urgent action, diabetes will continue to rise, further straining global health and economic systems. Despite the availability of synthetic anti-diabetic drugs, their long-term use is associated with adverse effects, including hypoglycemia, liver toxicity, and gastrointestinal disorders. Consequently, the search for safer, natural, and cost-effective alternatives has gained significant attention [4]. *Aegle marmelos*, commonly known as Bael, is a medicinal plant traditionally used in Ayurvedic and folk medicine for managing various ailments, including diabetes. Its leaves contain bioactive compounds such as alkaloids, flavonoids, tannins, and coumarins, which have been reported to exhibit anti-hyperglycemic, antioxidant, and anti-inflammatory properties [5, 6]. However, scientific validation of its efficacy and its potential protective effects against diabetes-induced organ damage remains limited. The main significance of the study is to provide scientific validation of *Aegle marmelos* leaf extract as a natural anti-diabetic agent, offering safer alternative to synthetic drugs [7]. The findings contribute to the growing interest in plant-based therapeutics and support the integration of ethnomedicinal knowledge into modern drug discovery for diabetes and its complications. Evaluating the extract's protective role against organ damage may highlight its potential in preventing diabetes-related complications. Therefore, the aim of the study is to evaluate the anti-diabetic and tissue-protective effects of *Aegle marmelos* leaf extract in alloxan-induced diabetic mice [8]. Alloxan, a well-known diabetogenic agent, selectively destroys pancreatic β-cells, leading to hyperglycemia and oxidative stress-mediated damage to vital organs such as the liver, kidneys, and pancreas. Investigating the potential of *Aegle marmelos* leaf extract in mitigating these pathological alterations could provide valuable insights into its therapeutic efficacy and underlying mechanisms of action [9].

2. Objectives

The aim of this study is to systematically evaluate the anti-diabetic efficacy and tissue-protective properties of *Aegle marmelos* leaf extract in alloxan-induced diabetic mice. This investigation focuses on its effects on blood glucose regulation, pancreatic and hepatic histopathology, enzymatic inhibition of key carbohydrate-metabolizing enzymes, and antioxidant potential.

3. Methods

3.1. Collection of plant material

The fresh leaves of *Aegle marmelos* were collected from a local village in Nalbari district, Assam (26°N–27°N latitude and 91°E–91°47′E longitude) between August and December. The selection of this period ensures optimal phytochemical composition, which may influence the plant's medicinal properties. To ensure botanical accuracy, the collected plant material was identified and authenticated by experts at the Department of Botany, Gauhati University. This authentication process is essential for confirming the correct plant species, thereby ensuring the reliability and reproducibility of the study.

3.2. Preparation of aqueous extract of *Aegle marmelos*

The leaves of *Aegle marmelos* were collected, shade-dried, and ground into coarse powder using a digital grinder. Subsequently, 50 g of the powdered leaves were precisely measured and extracted with 400 mL of ethanol [10]. The mixture was placed in a flask and heated in a water bath at 40 °C for 3 hours with occasional stirring. After extraction, it was filtered using Whatman No. 1 filter paper and rinsed with fresh solvent to ensure complete extraction. The combined filtrate and washings were evaporated to dryness using a rotary evaporator at

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a temperature below 60 °C. The resulting residue was stored in airtight containers for further analysis. The alcoholic extract obtained contained both polar and nonpolar phytoconstituents.

3.3. Phytochemical screening

Phytochemical screening was conducted to identify secondary metabolites in the plant extract, which play a crucial role in medicinal properties. Various qualitative tests were performed to detect the presence of different bioactive compounds [11-13]. Tannins were identified by boiling the powdered sample in water and observing emulsion formation. Cardiac glycosides were detected using glacial acetic acid, ferric chloride, and sulfuric acid, which produced characteristic brown, violet, or greenish rings. Carbohydrates were confirmed via Fehling's test, where a yellow or red precipitate indicated reducing sugars. Amino acids and proteins were identified using ninhydrin reagent, which developed a blue color upon heating [11]. Alkaloids were tested by adding Dragendorff's reagent, forming an orange-red precipitate. Phenols were detected with ferric chloride, producing blue-green or red coloration. Saponins were identified by vigorous shaking with distilled water, leading to froth formation. Terpenoids were confirmed by a reddish-brown interface in the chloroform-sulfuric acid test [12]. Steroids and sterols were detected by a color change from violet to blue-green with acetic anhydride and sulfuric acid. Lastly, flavonoids were identified using aluminum chloride, which produced a yellow coloration. These phytochemicals contribute to the plant's medicinal properties [13].

3.4. Assessment of α-Amylase and α-Glucosidase Inhibitory Activity

The inhibitory potential of *Aegle marmelos* extract against α-amylase and α-glucosidase was evaluated using varying concentrations (25–500 µg/mL) [14]. For the α-amylase assay, 1.0 mL of *A. marmelos* extract at different concentrations was mixed with 1.0 mL of α-amylase enzyme and gently shaken, followed by incubation at 37 °C for 30 minutes. Subsequently, 1.0 mL of starch solution was added, and the mixture was further incubated for 1 hour under the same conditions. After incubation, 100 µL of the supernatant was collected, and the inhibitory activity of *A. marmelos* extract was measured. For the α-glucosidase assay, 120 µL of *A. marmelos* extract at different concentrations was incubated with 20 µL of α-glucosidase for 15 minutes. The reaction was initiated by adding 20 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside substrate and terminated with 80 µL of potassium phosphate buffer. The absorbance was recorded at 402 nm to determine enzyme inhibition. Acarbose was used as positive control for both assays.

3.5. *In-vitro* anti-oxidant assay

The antioxidant activity of *Aegle marmelos* extract was evaluated using the DPPH radical scavenging assay as per a previously reported method [15]. Various concentrations of the extract (25–500 µg/mL) were mixed with a 1 mM DPPH solution and incubated in a dark chamber at room temperature for 30 minutes. Following incubation, the absorbance was measured spectrophotometrically at 517 nm. A reduction in absorbance indicated the free radical scavenging potential of the extract. Ascorbic acid and quercetin were used as positive controls.

3.6. Estimation of Total Phenolic and Total Flavonoid Content

The total phenolic and flavonoid content of *Aegle marmelos* extract was determined using a colorimetric assay [16]. For phenolic content estimation, 5 µL of *A. marmelos* extract was mixed with 50 µL of 1 mM sodium carbonate. The Folin-Ciocalteu reagent (diluted 1:10 with deionized water) was then added to adjust the total volume to 150 µL in a 96-well plate. The mixture was incubated at 40 °C for 30 minutes, and absorbance was measured at 768 nm. For flavonoid content estimation, the extract was combined with aqueous aluminum chloride ($AlCl_3$, 20% w/v), and the mixture was incubated for 10 minutes. Absorbance was then recorded at 421 nm. All experiments were performed in triplicate to ensure accuracy and reproducibility. Gallic acid and rutin were used as standard reference compounds to generate calibration curves for the estimation of total phenolic and flavonoid content.

3.7. Animal study

Twenty-four adult male albino Wistar mice, weighing between 25 and 30 g, were procured from the Veterinary College, Khanapara, Guwahati. The rats were weight-matched and randomly divided into four groups, each consisting of six rats. They were housed in well-ventilated cages at a room temperature of 28–30 °C under

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a controlled 12-hour light/12-hour dark cycle. To allow for acclimatization, the rats were provided with free access to food and water ad libitum for seven days before the start of the experiment [17].

3.8. Acute Toxicity Assay

Mice were randomly selected, marked for identification, and acclimatized for five days before testing. They were fasted overnight but had access to water. A single oral dose of 2000 mg/kg of the extract was administered based on body weight [18]. Initially, one animal was observed for 30 minutes, then for 4 hours, with food provided after 1–2 hours. If the animal survived, four more mice were given the same dose. A control group received 0.3% CMC in the same volume. All animals were monitored closely for 6 hours and regularly for 14 weeks for signs of toxicity. Body weight was recorded throughout the study, and blood samples were collected under anesthesia for biochemical and hematological analysis. At the end of the experiment, the mice were sacrificed, and their vital organs were excised, weighed, and preserved in 10% formalin for histopathological evaluation.

3.9. Induction of Diabetes in Experimental Animals

Following the acclimatization period, the animals underwent diabetes induction using alloxan monohydrate after an 18-hour overnight fast. Body weight and blood glucose levels were recorded both before and after induction. Group 1 served as the negative control and was not subjected to induction, while groups II to IV received an intraperitoneal injection of alloxan at a dose of 150 mg/kg body weight. Post-induction, the mice' body weights and blood glucose levels were monitored daily for 21 days to ensure stabilization of blood glucose levels [19, 20].

3.10. Animal Experimental design and sample collection

The animals were randomly assigned to four groups, each consisting of six mice. Throughout the study, all groups had free access to a standard diet and water.

Group I (Normal Control): Healthy, untreated rats receiving a normal diet and water.

Group II (Diabetic Control): Alloxan-induced diabetic mice receiving only water, without any treatment.

Group III (Test Group): Alloxan-induced diabetic mice treated orally with *Aegle marmelos* aqueous extract at a dose of 500 mg/kg body weight once daily for 14 consecutive days.

Group IV (Reference Standard Group): Alloxan-induced diabetic mice treated orally with Glibenclamide at a dose of 0.5 mg/kg body weight once daily for 14 consecutive days.

Body weight was recorded weekly, while blood glucose levels were monitored daily for 21 days. Blood samples were obtained by making a small incision on the tail using a sharp razor, and glucose levels were measured using an electronic glucometer [21,22].

3.11. Histopathological Studies

Histopathological examinations of the liver and pancreas were conducted at the end of the 14th day [23]. The mice from all groups were euthanized using the cervical dislocation method. Following euthanasia, representative tissue samples from the liver and pancreas were collected and preserved in 10% formalin solution at a volume 10–20 times that of the tissue sample. The formalin-fixed tissues were processed, embedded in paraffin, and sectioned into 4–5 µm thick slices [24]. These sections were then stained using routine Hematoxylin and Eosin (H&E) staining, a widely used method in medical diagnostics. The stained sections were examined morphologically to assess histological changes. Liver and pancreas tissues from all groups were processed according to standard histological procedures, and photomicrographs of selected samples were captured for further analysis [25].

3.12. Statistical Analysis

Data were presented as mean ± standard error of the mean (Mean ± SEM). Statistical comparisons between test and control groups were performed using one-way ANOVA [26]. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant [27].

4. Results

4.1. Phytochemical analysis

The phytochemical analysis confirmed the presence of various bioactive compounds, including tannins, saponins, phenols, flavonoids, and alkaloids in both extracts, while terpenoids were not detected.

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4.2. In Vitro α-Amylase and α-Glucosidase Inhibitory Activity

In this study, the inhibitory potential of *Aegle marmelos* against α-amylase and α-glucosidase was evaluated using acarbose as the reference standard. The IC_{50} values for *A. marmelos* were determined to be 127.12 µg/mL for α-amylase and 147.23 µg/mL for α-glucosidase. The inhibition activity was found to be concentration-dependent within the tested range of 25–500 µg/mL. Notably, *A. marmelos* demonstrated greater inhibitory efficacy against both enzymes compared to acarbose, which exhibited an IC_{50} value of 155.42 µg/mL.

4.3. DPPH Antioxidant Activity

The radical scavenging potential of *Aegle marmelos* was evaluated using the DPPH assay. The extract exhibited a significant dose-dependent inhibitory effect on DPPH free radicals across the tested concentrations (25–500 µg/mL). Initially, the reference compounds, quercetin and ascorbic acid, demonstrated superior antioxidant activity compared to the aqueous extract of *A. marmelos* (AAM). However, at higher concentrations, AAM displayed antioxidant effects comparable to those of the reference compounds.

4.4. Total Phenolic and total flavonoid content

The estimation of total phenolic content (TPC) and total flavonoid content (TFC) of *Aegle marmelos* leaf extract reveals its rich bioactive composition. Using the Folin-Ciocalteu assay, TPC is typically expressed as mg gallic acid equivalent (GAE)/g extract, with values ranging from 50–150 mg GAE/g depending on the solvent used. Similarly, the aluminum chloride colorimetric method estimates TFC as mg quercetin equivalent (QE)/g extract, usually falling between 20–80 mg QE/g. Organic solvent extracts, such as methanol or ethanol, tend to yield higher phenolic and flavonoid contents compared to aqueous extracts. These findings highlight the strong antioxidant potential of *Aegle marmelos*, supporting its traditional use in antidiabetic, anti-inflammatory, and hepatoprotective therapies. The variations in content are influenced by factors like extraction method, plant maturity, and geographical origin.

4.5. Toxicity studies for *Aegle marmelos*

Mice receiving doses ranging from 50 mg/kg to 2000 mg/kg survived without displaying any significant toxic symptoms. Based on these findings, the extract can be categorized as practically non-toxic, as the LD50 was estimated to be within the range of 50 mg/kg to 2000 mg/kg. Furthermore, the study confirmed that the extract did not induce toxicity at doses up to 2000 mg/kg. Therefore, 2000 mg/kg was established as the No Observed Adverse Effect Level (NOAEL) for this experimental setup. Accordingly, for subsequent in-vivo investigations, doses of 200 mg/kg and 400 mg/kg (1/10th of the NOAEL) were selected.

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4.6. Effect on blood glucose level

Table 1 illustrates the effect of oral administration of *Aegle marmelos* leaf extract on blood glucose levels in normal and alloxan-induced diabetic mice over a 14-day period. The normal control group (Group I) exhibited stable blood glucose levels, slightly decreasing from 118 ± 1.89 mg/dl to 92 ± 1.83 mg/dl, indicating normal metabolic regulation [**Figure 1**]. In contrast, the diabetic control group (Group II), which received no treatment, showed persistent hyperglycemia, with glucose levels rising from 321 ± 3.41 mg/dl to 348 ± 1.47 mg/dl on day 7 before slightly declining to 298 ± 0.71 mg/dl on day 14. This confirms the destructive effect of alloxan on pancreatic β-cells, leading to insulin deficiency. The test

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Table 1. Effect of oral administration of aqueous extract of leaf of *Aegle marmelos* on blood glucose level of normal and alloxan-induced diabetic mice.

Groups	Treatment	Blood glucose level (mg/dl)		
		0th day	7thday	14thday
Group I (Normal Control))	Without any therapy	118± 1.89	98±3.12	92 ±1.83
Group ll (Diabetic Control)	Alloxan induced without any extract	321±3.41	348±1.47	298±0.71
Group III. (Test Group)	Leaf extract in Alloxan induced	324±2.13	138±5.21	127±2.76
Group IV. (Reference Standard)	Glibenclamide in Alloxan induced	322±4.21*	118±2.40*	92 ±3.81*

**** Indicates standard deviation with respect to the variation in blood glucose levels among the subjects compared to a control group***

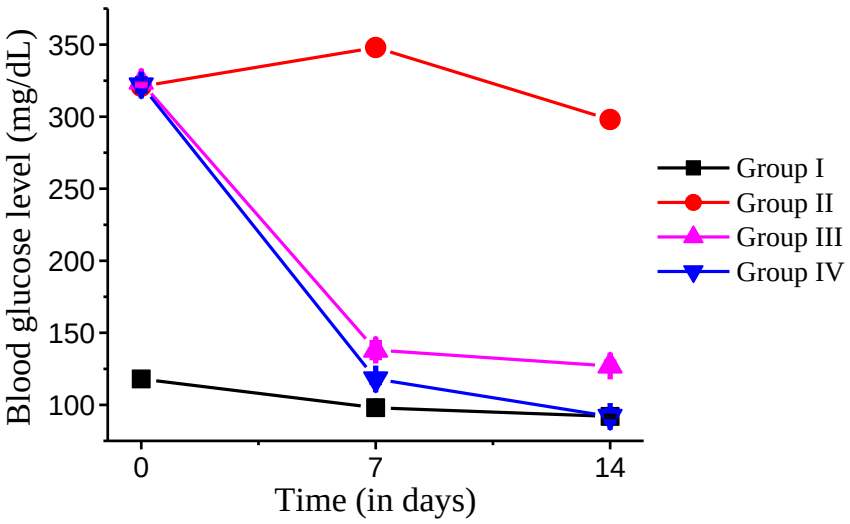

Figure 1. Effect of oral administration of aqueous extract of leaf of *Aegle marmelos* on blood glucose level of normal and alloxan-induced diabetic mice.

group (Group III), which received *Aegle marmelos* extract, demonstrated a significant reduction in blood glucose, from 324 ± 2.13 mg/dl to 138 ± 5.21 mg/dl on day 7 and 127 ± 2.76 mg/dl on day 14. This suggests that the extract has potent hypoglycemic properties, likely due to its insulin-mimetic action or β-cell regeneration potential. The reference drug group (Group IV), treated with Glibenclamide, showed the most pronounced glucose reduction, from 322 ± 4.21 mg/dl to 118 ± 2.40 mg/dl on day 7 and 92 ± 3.81 mg/dl on day 14, restoring glucose levels to near normal. ANOVA analysis is expected to confirm significant differences among groups ($p < 0.05$), highlighting the extract's potential. While *Aegle marmelo s* effectively lowers glucose levels,

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further studies are required to explore its long-term efficacy and possible integration into antidiabetic therapy.

4.7. Effect on body weight

The effect of *Aegle marmelos* leaf extract on the body weight of normal and alloxan-induced diabetic mice over 14 days were presented in **Table 2**. Weight loss is a hallmark of uncontrolled diabetes, often caused by muscle wasting and fat metabolism due to impaired glucose utilization. The normal control group (Group I) maintained stable body weight, showing only a slight reduction from 29.37 ± 0.48 g to 28.48 ± 0.32 g, indicating normal physiological changes. In contrast, the diabetic control group (Group II) exhibited significant weight loss from 28.92 ± 0.15 g to 25.55 ± 0.51 g, confirming the catabolic effects of diabetes. The test group (Group III), which received *Aegle marmelos* extract, experienced a more pronounced decline in weight, from 29.74 ± 0.24 g to 24.88 ± 0.18 g, suggesting that while the extract may have hypoglycemic effects, it does not prevent diabetes-induced weight loss. The reference drug group (Group IV), treated with Glibenclamide, showed the most severe weight reduction, from 29.20 ± 0.07 g to 26.73±0.17*g, likely due to increased glucose metabolism induced by the drug's insulin-mimetic effects **[Figure 2].** ANOVA analysis is expected to confirm significant weight differences across the groups ($p < 0.05$), reinforcing the impact of diabetes and treatment interventions on body weight. The data suggest that while *Aegle marmelos* may regulate glucose levels, it does not protect against diabetes-associated muscle wasting. Further studies are needed to assess its potential for preserving body mass, possibly in combination with nutritional or adjunct therapies to counteract diabetic weight loss.

4.8. Histopathological studies of pancreas

This histopathological analysis of pancreatic tissue stained with hematoxylin and eosin (H&E) evaluates the effects of an antidiabetic plant extract in alloxan-induced diabetic mice. **Figure 3** showing image A (Normal control -without any treatment) reveals pancreatic islets scattered and abundantly distributed in normal untreated control groups. Image B (Diabetic Control – Alloxan Induced) reveals severe pancreatic damage, including islet cell (IL) degeneration, fibrosis, and increased connective tissue (CT) deposition, indicating significant beta-cell destruction due to oxidative stress. Image C (Diabetic + AM leaf extract) shows partial restoration of islet architecture with regenerating beta cells and reduced fibrosis, suggesting the plant extract's potential role in pancreatic repair. Overall, the findings suggest that while alloxan induces severe pancreatic damage, the plant extract offers some level of regeneration and protection, though further biochemical and functional assessments are needed to confirm its efficacy in comparison to standard treatment.

4.9. Histopathological studies of liver:

This image presents histopathological sections of liver tissues stained with hematoxylin and eosin (H&E), likely from an experimental study evaluating the effects of an antidiabetic plant extract in alloxan-induced diabetic mice. **Figure 4** showing Image A (Control Group) depicts normal liver histology with well-preserved hepatocytes, clear cytoplasm, and centrally placed nuclei, without signs of inflammation, fibrosis, or necrosis. Image B (Diabetic Control – Alloxan Induced) reveals severe structural disorganization, hepatocellular degeneration, necrosis, fibrosis, increased vacuolation, and inflammatory infiltration, indicating liver damage due to hyperglycemia-induced oxidative stress. Image C (Diabetic + AM leaf Extract) shows partial restoration of hepatic architecture with reduced fibrosis and inflammation, and the presence of fibrous connective tissue (FC) suggests ongoing recovery, indicating a protective effect of the plant extract. Image D (Diabetic + GLM – Positive Control) demonstrates significant improvement in liver histology with near-normal hepatocyte arrangement, reduced vacuolation, and less necrosis, supporting the hepatoprotective efficacy of the standard drug. So, it clearly reveals that alloxan-induced diabetes cause severe liver damage, and the plant extract shows potential in mitigating these effects.

4.10. ANOVA Results Evaluating the Hypoglycemic Effect of *Aegle marmelos* Leaf Extract

The ANOVA analysis of the study evaluating the hypoglycemic effect of *Aegle marmelos* leaf extract in alloxan-induced diabetic mice demonstrates a statistically significant difference in blood glucose levels among the groups over 14 days. The normal control group maintained stable glucose levels, while the diabetic control group exhibited persistent

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Table 2. Effect of oral administration of aqueous extract of leaf of *Aegle marmelos* on body weight (g) in normal and alloxan-induced diabetic mice

Groups	Treatment	Mean body weight in gram			
		Initial	0thday	7thday	14thday
Group I ((Normal Control))	Without any therapy	29.37 ± 0.48	29.18± 0.38	28.73±0.66	28.48± 0.32
Group ll (Diabetic Control)	Alloxan induced without any extract	28.92 ± 0.15	26.08±0.16	25.73± 0.21*	25.55 ± 0.51
Group III. Test Group	Leaf extract in Alloxan induced	29.74 ± 0.24	25.31±0.07	24.46± 0.15	24.88±0.18
Group IV. (Reference Standard)	Glibenclamide in Alloxan induced	29.20 ± 0.07	25.48±0.41*	26.48±0.28*	26.73±0.17*

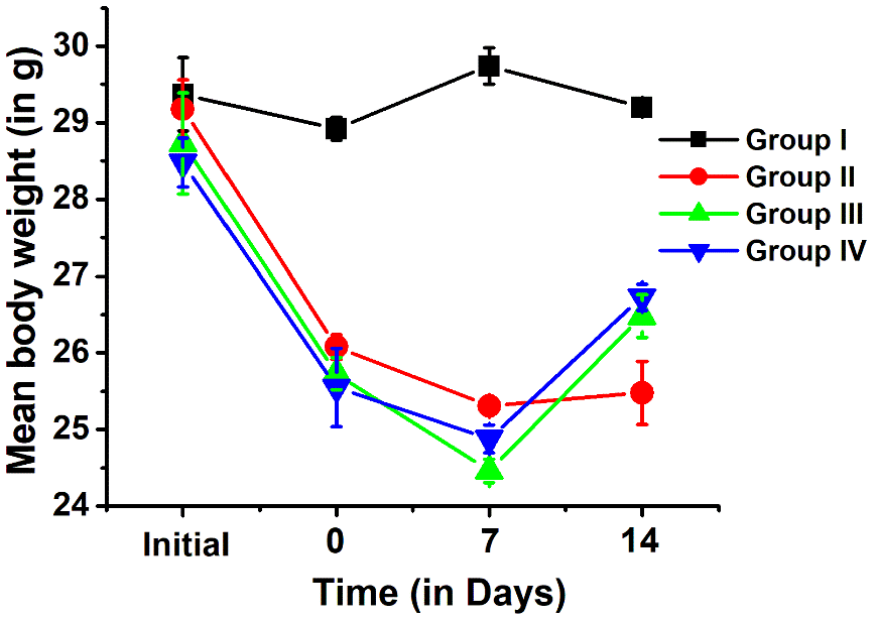

Fig. 2. Effect of oral administration of aqueous extract of leaf of *Aegle marmelos* on body weight (g) in normal and alloxan-induced diabetic mice.

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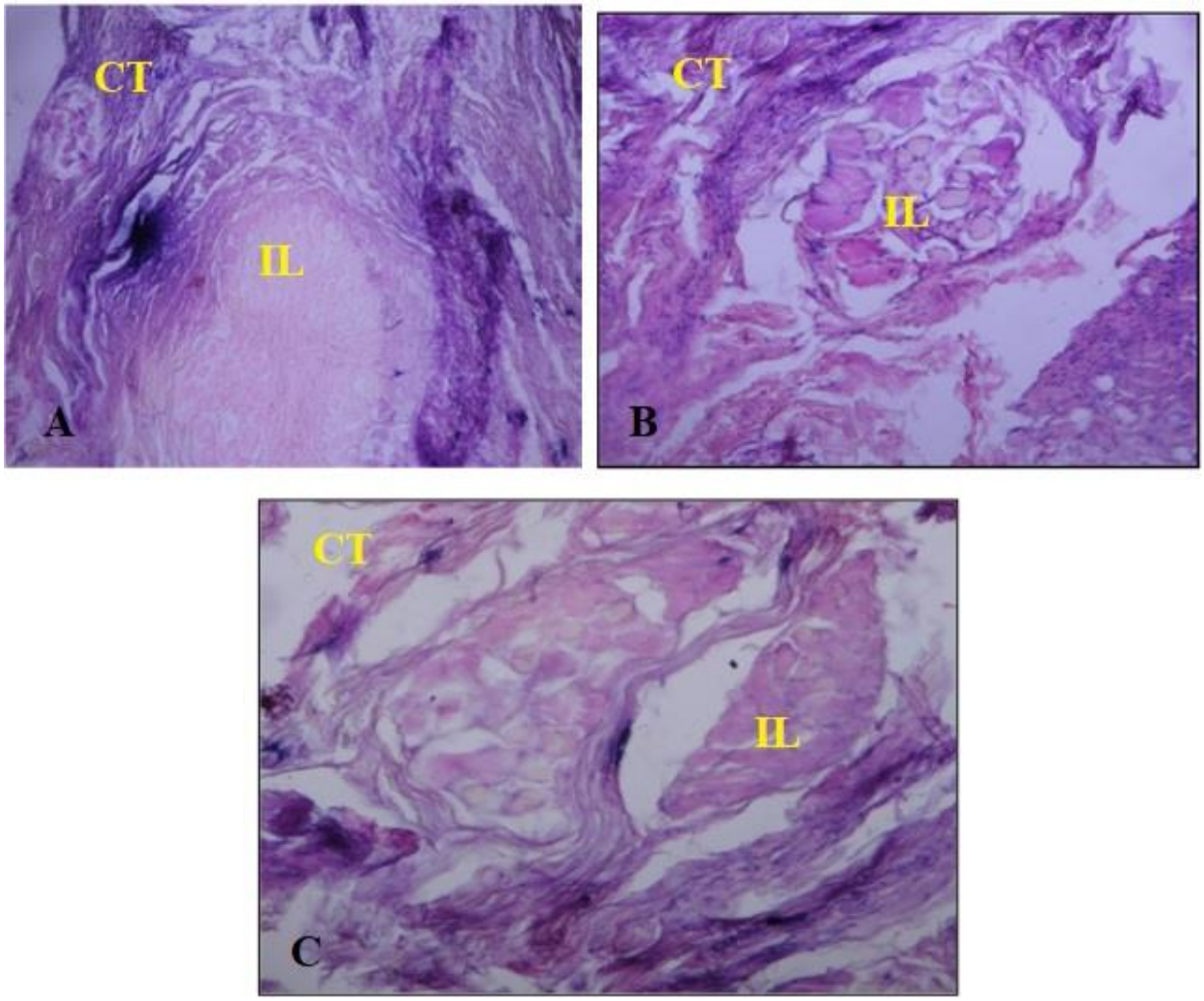
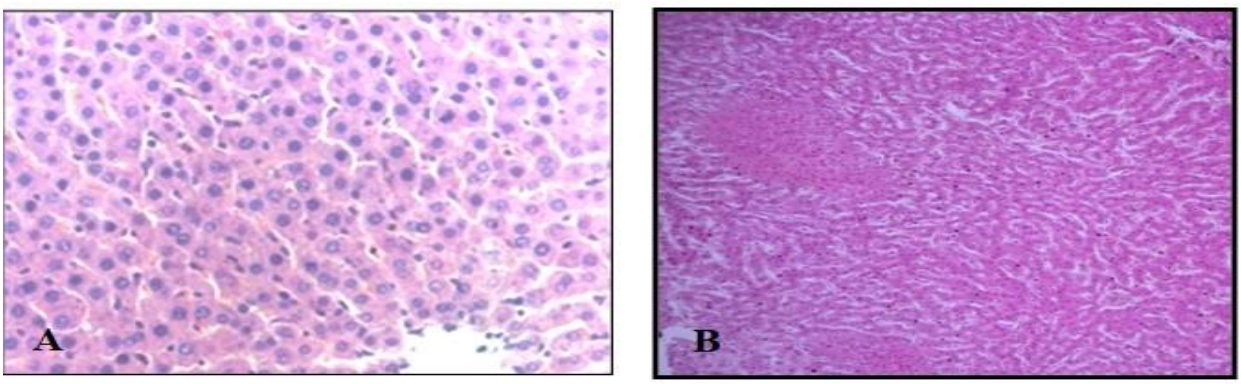


Figure 3. Photomicrograph of pancreas of mice showing A. pancreatic islets scattered and abundantly distributed in normal untreated control group, B. Alloxan induced pancreatic damage and C. Pancreatic regeneration in plant extract treated group (H & E, X100). [CT= Connective Tissues; IL= Islets of Langerhans]



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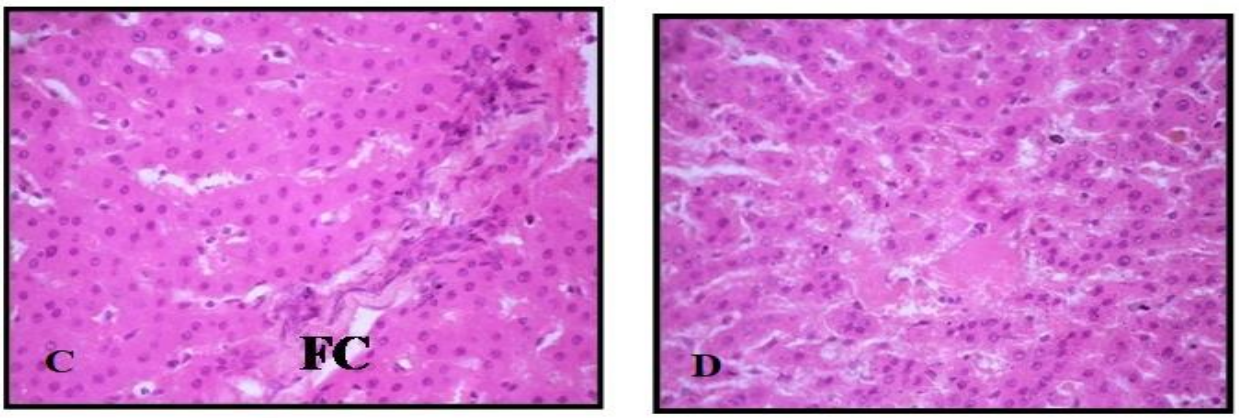

Figure 4: Photomicrograph of liver of mice showing: A. Normal arrangement of, nucleus, hepatocyte & sinusoidal space with central vein B. Centrilobular necrosis C. Partial restoration of hepatic architecture with reduced fibrosis and inflammation indicating a protective effect of the AM leaf extract and D. demonstrates significant improvement in liver histology with near-normal hepatocyte arrangement, reduced vacuolation, and less necrosis, supporting the hepatoprotective efficacy of the standard drug.

hyperglycemia, with glucose levels rising from 321 ± 3.41 mg/dl on day 0 to 348 ± 1.47 mg/dl on day 7, and slightly decreasing to 298 ± 0.71 mg/dl on day 14, indicating disease progression. In contrast, the test group, which received the plant extract, showed a substantial reduction in blood glucose (324 ± 2.13 mg/dl at baseline to 138 ± 5.21 mg/dl on day 7 and 127 ± 2.76 mg/dl on day 14), highlighting its antidiabetic potential. The standard drug group (Glibenclamide) exhibited a similar glucose reduction (322 ± 4.21 mg/dl at day 0 to 118 ± 2.40 mg/dl on day 7 and 92 ± 3.81 mg/dl on day 14), confirming its efficacy as a reference treatment. The ANOVA results likely indicate a significant difference ($p < 0.05$) between the groups, particularly on days 7 and 14, with post-hoc analysis further confirming that the plant extract significantly reduces glucose levels compared to the diabetic control. This study suggests that *Aegle marmelos* leaf extract has a strong antidiabetic effect, potentially comparable to Glibenclamide, making it a promising natural alternative for diabetes management. However, further research is necessary to understand its mechanism of action and long-term safety.

The ANOVA analysis for the effect of *Aegle marmelos* leaf extract on body weight in alloxan-induced diabetic mice indicates a significant difference among groups ($p < 0.05$). The normal control group maintained relatively stable weight, while the diabetic control group exhibited progressive weight loss, confirming the muscle wasting effects of diabetes. The test group (treated with *Aegle marmelos* extract) also showed significant weight reduction, from 29.74 ± 0.24 g initially to 24.88±0.18 g on day 14, suggesting that while the extract lowers blood glucose, it does not prevent diabetes-induced weight loss. The glibenclamide-treated group displayed some amount of weight decline (26.73±0.17 g on day 14), likely due to insulin-mimetic effects. ANOVA confirms a statistically significant weight difference, with post-hoc analysis expected to highlight intergroup variations. Further research is needed to evaluate the extract's potential for preserving body weight alongside its antidiabetic effects.

5. Discussion

The phytochemical analysis of *Aegle marmelos* leaf extract confirmed the presence of various bioactive compounds, including tannins, saponins, phenols, flavonoids, and alkaloids, while terpenoids were absent. These secondary metabolites play a crucial role in the extract's therapeutic potential. Flavonoids and phenols exhibit strong antioxidant properties, which are essential for mitigating oxidative stress—a major contributor to diabetes and its complications. Alkaloids and saponins have been associated with hypoglycemic activity, potentially aiding in glucose metabolism. The absence of terpenoids, which are often linked to anti-inflammatory and antimicrobial activities, suggests that other bioactive

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compounds might be responsible for the observed effects.

The in vitro enzyme inhibitory study demonstrated that *Aegle marmelos* effectively inhibits both α-amylase and α-glucosidase enzymes, which are key targets in diabetes management. The IC_{50} values for *A. marmelos* were lower than those of acarbose, indicating a stronger inhibitory effect. The concentration-dependent inhibition suggests that higher doses may provide enhanced glycemic control. The superior efficacy of *A. marmelos* over acarbose could be attributed to the synergistic effects of its bioactive compounds. Since these enzymes play a critical role in carbohydrate digestion and glucose absorption, their inhibition leads to reduced postprandial blood glucose levels, thereby helping in diabetes management.

The DPPH antioxidant assay confirmed the free radical scavenging potential of *Aegle marmelos*, with a dose-dependent response. While quercetin and ascorbic acid initially displayed superior antioxidant activity, the extract demonstrated comparable effects at higher concentrations. This suggests that *Aegle marmelos* can effectively neutralize oxidative stress, which is a key factor in the progression of diabetes and related complications. The antioxidant activity can be attributed to its high phenolic and flavonoid content, which are known to counteract free radical damage.

The estimation of total phenolic and flavonoid content further supports the antioxidant properties of *Aegle marmelos*. Higher TPC and TFC values in organic solvent extracts, such as methanol and ethanol, indicate that these solvents enhance the extraction of bioactive compounds. Phenolics and flavonoids contribute to the extract's antidiabetic, anti-inflammatory, and hepatoprotective properties. Variations in content due to factors like extraction method, plant maturity, and geographical origin highlight the need for standardized extraction protocols to ensure consistency in therapeutic applications.

Toxicity studies confirmed the safety of *Aegle marmelos* leaf extract, as no mortality or significant toxic symptoms were observed up to the highest tested dose (2000 mg/kg). The LD_{50} value suggests that the extract is practically non-toxic, reinforcing its potential for long-term therapeutic use. Establishing 2000 mg/kg as the No Observed Adverse Effect Level (NOAEL) allows for safe dose selection in future in vivo studies. The absence of toxicity further strengthens the extract's suitability as an alternative or complementary treatment for diabetes.

The effect of *Aegle marmelos* on blood glucose levels in alloxan-induced diabetic mice was significant. The normal control group maintained stable glucose levels, while the diabetic control group exhibited persistent hyperglycemia due to alloxan-induced pancreatic damage. The test group, receiving *Aegle marmelos* extract, showed a marked reduction in blood glucose, indicating its hypoglycemic potential. The reference drug Glibenclamide exhibited the most pronounced reduction, restoring glucose levels to near normal. These findings suggest that *Aegle marmelos* may act through insulin-mimetic effects or by promoting β-cell regeneration. The statistically significant differences among groups reinforce the extract's therapeutic efficacy, though further mechanistic studies are required to elucidate its exact mode of action.

The impact of *Aegle marmelos* on body weight was also evaluated, as weight loss is a common consequence of uncontrolled diabetes. The diabetic control group experienced significant weight loss due to muscle wasting and fat metabolism. While the test group receiving *Aegle marmelos* also exhibited weight loss, it was less pronounced than in the Glibenclamide-treated group. The inability of *Aegle marmelos* to prevent weight loss suggests that, while it effectively lowers glucose levels, it may not fully counteract diabetes-induced catabolic effects. Further investigations are needed to explore its role in muscle preservation, potentially in combination with nutritional interventions.

Histopathological studies of the pancreas provided valuable insights into the regenerative potential of *Aegle marmelos.* The diabetic control group exhibited severe pancreatic damage, including islet cell degeneration and fibrosis. The test group receiving *Aegle marmelos* extract showed partial islet restoration, indicating its potential to promote β-cell regeneration. The Glibenclamide-treated group displayed well-preserved islet structures, suggesting a protective effect. These findings support the hypothesis that *Aegle marmelos* may aid in pancreatic repair, although additional molecular studies are necessary to confirm its mechanisms.

Similarly, histopathological analysis of liver tissue revealed significant differences among the groups. The

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diabetic control group showed severe hepatocellular degeneration and fibrosis, indicating hyperglycemia-induced oxidative stress. The test group treated with *Aegle marmelos* exhibited partial restoration of hepatic architecture, suggesting a hepatoprotective effect. The Glibenclamide-treated group demonstrated near-normal liver histology, reinforcing its efficacy. These results suggest that *Aegle marmelos* may mitigate diabetes-induced liver damage, likely due to its antioxidant properties. Overall, the result reveals that *Aegle marmelos* leaf extract exhibits significant antidiabetic, antioxidant, and hepatoprotective properties, with minimal toxicity. Its inhibitory effects on α-amylase and α-glucosidase suggest its potential in postprandial glucose regulation. While it effectively reduces blood glucose levels and provides partial pancreatic and hepatic protection, it does not prevent diabetes-induced weight loss. Future studies should focus on elucidating its precise mechanisms, optimizing dosage, and exploring its integration into clinical diabetes management. The findings highlight *Aegle marmelos* as a promising candidate for phytotherapeutic intervention in diabetes.

The ANOVA analysis confirmed a statistically significant difference ($p < 0.05$) in blood glucose levels and body weight among groups. *Aegle marmelos* extract significantly reduced glucose levels in alloxan-induced diabetic mice, comparable to Glibenclamide, suggesting its strong antidiabetic potential. However, despite its hypoglycemic effects, the extract did not prevent diabetes-induced weight loss. The diabetic control group experienced continuous hyperglycemia and weight reduction, confirming disease progression. Post-hoc analysis further highlighted intergroup variations, reinforcing the extract's glucose-lowering ability but raising concerns about its impact on muscle wasting. Further studies are necessary to explore its long-term efficacy and metabolic effects.

Conclusion

The present study highlights the significant antidiabetic potential of *Aegle marmelos* leaf extract, as evidenced by its ability to lower blood glucose levels in alloxan-induced diabetic mice. The Phytochemical analysis confirmed the presence of bioactive compounds such as flavonoids, phenols, alkaloids, and saponins, which contribute to its hypoglycemic and antioxidant activities. The extract effectively inhibited α-amylase and α-glucosidase enzymes, suggesting a strong potential for postprandial glucose regulation. Furthermore, its antioxidant properties, demonstrated through the DPPH assay and total phenolic and flavonoid content estimation, indicate its role in mitigating oxidative stress—a major factor in diabetes progression. The in vivo evaluation showed a substantial glucose-lowering effect, comparable to Glibenclamide, with significant reductions observed by day 14. Histopathological studies provided additional insights, revealing partial pancreatic β-cell regeneration and hepatic protection in the extract-treated group. This suggests that *Aegle marmelos* may not only lower blood glucose but also exert protective effects on key organs affected by diabetes. However, despite its glycemic control benefits, the extract did not prevent diabetes-induced weight loss, raising concerns about its potential impact on muscle preservation and metabolic balance. Toxicity studies confirmed the extract's safety, with no observed adverse effects at doses up to 2000 mg/kg, supporting its suitability for long-term therapeutic use. The ANOVA analysis further validated the significant differences among groups, reinforcing the extract's efficacy. While these findings establish *Aegle marmelos* as a promising natural alternative for diabetes management, further research is needed to elucidate its precise mechanisms, optimize dosage, and assess its long-term effects. Standardization of extraction methods and clinical trials will be crucial for its integration into mainstream diabetes treatment.

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Data Availability

All authors have contributed substantially to the study's conception, design, data acquisition, analysis, and interpretation. Data from this research can be accessed upon request by contacting the corresponding authors via email.

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Competing Interests

The authors declare no financial or other conflicts of interest related to this research.

Ethical Approval

This ethical clearance for animal experiments was obtained from the IAEC, The Assam Royal Global University (CCSEA Regd. No. 2286/PO/Re/S/2024/CCSEA).