Bima Journal of Science and Technology, Vol. 8(3B) Oct, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i3B.848

Phytotherapeutic Evaluation of *Psidium guajava* Leaf Extract on Glycemic Control and Hepatic Function in Alloxan-Induced Diabetic Mice

[1]Ahmad Aliyu Ladan, [2]Abdul-kareem Yusuf Idris, [1]Abubakar Khadija Zirami, [1]Ahmad Aminu Muhammad, [1]Habib Musa Jibrin, and [1*]Abubakar Khalid Aliyu

[1]Department of Physiology Sa'adu Zungur University Bauchi, Bauchi State
[2]Department of veterinary Physiology Pharmacology and Biochemistry University of Jos

Corresponding Author: abukhalid6603@gmail.com

ABSTRACT

Diabetes mellitus (DM) is one of the most prevailing metabolic disorders, characterized by high level of blood glucose level, which may result from either the pancreas's failure to secrete insulin or the inability of insulin receptors to respond to insulin. *Psidium guajava* shows promise as effective therapeutic option for this disease management. This study investigated the ameliorative effects of *Psidium guajava* aqueous leaf extract on blood glucose levels and liver damage in mice with alloxan-induced diabetes (120 mg/kg). Thirty mice (20-35g) were divided into six groups of five. Group I (non-diabetic) received normal saline. Diabetic groups II-VI received: normal saline (II), Metformin (5 mg/kg, III), and *Psidium guajava* aqueous extract at 150 mg/kg (IV), 300 mg/kg (V), and 450 mg/kg (VI). Diabetes induction was confirmed 72 hours post-administration. Oral gavage administration was conducted daily for a period of 28 days. Blood glucose was estimated on the 14th and 28th day of administration. On the 28th day, animals were euthanized, and blood samples were collected for biochemical analysis. This study's results revealed significant reductions ($P<0.05$) in liver enzymes (ALT, AST) and blood glucose levels compared to controls, indicating potential hepatoprotective and hypoglycemic effects. The results of this research indicate that *Psidium guajava* exhibits hepatoprotective effect and alleviate hyperglycemia and may mitigate diabetes-induced hepatic disorders.

Keywords: Diabetes mellitus, *Psidium guajava*, Alloxan, Liver.

INTRODUCTION

Diabetes mellitus (DM) is one of the most prevailing metabolic disorders, characterized by high level of blood glucose level, which may result from either the pancreas's failure to secrete insulin or the inability of insulin receptors to respond to insulin (Eze and Maxwell, 2021). The prevalence of diabetes mellitus according to IDF, has reached 415 million adults globally, with forecasts predicting a significant increase to 642 million by 2040 (Yang et al., 2020). Concurrently, direct medical costs are nearing the $1 trillion mark and are projected to escalate beyond this point by 2030 (Chu et al., 2022). In the 20-year period 2000 to 2019, diabetes was responsible for 4,509,047 deaths (53.2% in women) (Antini et al., 2024). Contrary to type 1 diabetes, a condition characterized by immune-mediated devastation of pancreatic beta cells, leading to insulin deficiency, the prevalence of type 2 diabetes mellitus has been increasing steadily (Eze and Maxwell, 2021). In contrast, T2DM has emerged as a more significant concern in developing nations due to urbanization and the resulting lifestyle changes, particularly the adoption of a Western-style diet high in fat (Yang et al., 2020).

Type 2 diabetes (T2D) is marked by impaired insulin sensitivity in key tissues (liver, muscles, and adipose) and/or diminished

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insulin production by pancreatic β-cells, resulting in hyperglycemia. This, in turn, triggers severe complications, including microvascular (retinopathy, neuropathy, nephropathy) and macrovascular (atherosclerosis, cardiovascular disease) disorders (Beidokht et al., 2020). Various experimental agents have been examined for their significant effects on different types of diabetes. Traditional medicine plays a vital role in reducing morbidity, and nearly 80% of the global population relies on herbal remedies due to growing awareness of the adverse effects and toxic tissue loading caused by conventional drugs (Abu Elez et al., 2021). One such plant used in diabetes treatment is *Psidium guajava*, commonly known as guava in English and Gwaiba in Hausa. The root, bark and leaves of *Psidium guajava* have been employed in traditional medicine for centuries to treat a wide range of health issues, including diabetes, cardiovascular disease, cancer, gastrointestinal disorders (gastroenteritis, vomiting, dysentery, diarrhea), wounds, dental problems (toothache, ulcers), respiratory issues (cough, sore throat), and oral health concerns (swollen gums), among others (Rajput and Kumar, 2021).

Studies have confirmed that aqueous extracts from guava leaves exhibit anti-hyperglycemic properties, effectively combating type 2 diabetes. Rich in antioxidants, antibacterial, anti-inflammatory, and hypoglycemic compounds, guava leaves show promise as a natural therapeutic agent for managing type 2 diabetes. Notably, diabetes remains a leading cause of mortality, with a 5% rise in premature deaths between 2000 and 2016, accounting for 1.6 million deaths in 2016 and 4.2 million deaths in 2019, emphasizing the need for effective treatments (Yusuf et al., 2024). The aim of goal study was, therefore, is to evaluate whether or not *psidium guajava* aqueous leaf extract can ameliorate hypoglycemia and the alteration induced by high level of glucose on the liver of male diabetic Swiss albino mice.

MATERIALS AND METHODS

Drugs, Chemicals, and Other Materials

All chemicals and drugs utilized in this study were obtained from commercial sources and met analytical-grade standards. The materials used included alloxan monohydrate, acquired from Puritan's Pride Inc. (Ronkonkoma, New York, USA), a digital glucometer (Accu-Check Advantage, Roche Diagnostic, Germany) for measuring blood glucose, and metformin, which was purchased from Xi'an ZB Biotech Co., Ltd, China.

Plant Materials

Fresh leaves of *Psidium guajava* was collected from the Botanical Garden of University of Jos, Jos, Plateau state on June, 2024. It was identified by Abdulkareem Yusuf Idris. The leaves of the plant were then air dried at room temperature for the period of 10 to 15 days.

Extraction of Plant Material

The dried leaves was crushed and size reduced to fine powdered using mortar and pestle, 75.78g of the powdered was cold macerated with 350ml of water for three days and shacked at regular interval of 4hour/day. The extract was filtered and concentrated with rotary evaporator at temperature of 40°C. The concentrated extract was heated with the water bath. The solvent free extract was later stored in the refrigerator at temperature of 4°C.

Phytochemical Screening

Phytochemical screening of the aqueous leaf extract of *Psidium guajava* was carried out by using standard procedure describe by (Sofowora, 1984).

Animals

30 normal healthy 20-35gm mice were obtained from the animal experimental unit of

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university of Jos, Jos Plateau State, Nigeria were used for this study. The mice were kept in plastic cages with stainless steel wire mesh cover, and maintained at relative humidity and temperature of about 25°C 12/12-hour light and dark circle. They were allowed to stay for a week for the purpose of acclimatization and possible detection of ill symptoms prior to the commencement of the experiment. The animal were fed with poultry starter feeds and drinking water. All aspect of animals' care complied with the ethical guidelines and technical requirements approved by the institutional animals' ethics committee were maintained.

Acute Toxicity Test

Lorke's method of 1983 was adopted in the LD, test of *Psidium guajava* (guava). This test is carried out by two phases. In the first phase, nine rats randomize in to three groups of three rats par group, 10, 100, 1000mg/kg body weight of the well-prepared extract of *Psidium guajava* (guava) was orally administered. The animals were observed at a very first four hours for possible detection of toxicity and subsequently 24 hours. The same procedure used in phase one was adopted in phase two but with different dose levels of 4000, 2000, 1000, 500 mg/kg body weight. The behavior of the mice was observed continuously for 1h, 4hrs and then intermittently for 24hrs after the treatment for any sign of toxicity and mortality.

Induction of Diabetes

Diabetes was administered using alloxan monohydrate. After an overnight fast, the rats were weighed, and their baseline glucose levels were recorded. The animals were then injected intraperitoneally with a single dose of alloxan (120 mg/kg body weight) following the method outlined by Eze and Maxwell (2021), after which they resumed their normal feeding schedule. Seventy-two hours post-induction, fasting blood glucose was measured using glucose oxidase principle. Blood glucose levels were determined using an On Call Plus digital glucometer, with fasting blood glucose levels of 120 mg/dl or higher regarded as diabetic.

Experimental Design

30 Swiss albino mice weighing between 20-35g were randomly selected into six groups (5 per cage);

- Group I: Normal control received 0.5ml normal saline
- Group II: Diabetic control received 0.5ml normal saline
- Group III: Standard drug (50 mg/kg b.w)
- Group IV: mice with diabetes treated with *psidium guajava* extract (150 mg/kg)
- Group V: mice with diabetes treated with *psidium guajava* extract (300 mg/kg)
- Group VI: mice with diabetes treated with *psidium guajava* extract (450 mg/kg)

Guava Leaves Mode of Administration

Guava leaf extract was dissolved in physiological saline and administered orally to Groups IV, V, and VI using an oro-gastric cannula at doses of 150 mg/kg, 300 mg/kg, and 450 mg/kg body weight, respectively, between 9:00 and 10:00 a.m. daily for up to four weeks. Additionally, Group III mice received 50 mg/kg body weight of metformin. Groups I and II (n=5) were not given either metformin or guava leaf extract.

Determination of alanine and aspartate aminotransferase activities

Serum ALT and AST levels were quantified using an automated biochemical analyzer and standardized assay kits, in accordance with the IFCC guidelines outlined in 2018.

Histological study

At the end of the treatment period, the animals were anesthetized with chloroform and euthanized by cervical dislocation. The

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liver was carefully collected from each mouse following standard procedures. The organs were thoroughly washed with 0.9% normal saline to eliminate any traces of blood and then preserved in formalin. Fat tissues attached to the organs were carefully removed, and the organs were sliced into small pieces using a surgical scalpel to ensure better chemical penetration into the tissues (Mamun et al., 2015).

Ethical clearance

The ethical clearance and permission to undertake this research were granted by the Research Ethics Committee, College of Medicine, University of Jos, Jos Plateau State, Nigeria. The experiment was conducted in accordance with the regulations of the Institutional Animal Care and Use (IACU) in collaboration with the Office of Laboratory Animal Welfare (OLAW) Reference number: F17-00379, University of Jos, Jos Plateau State, Nigeria.

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (SEM). The data were analyzed using one way analysis of variance ANOVA followed by Dunnet multiple comparison test. The value of $p < 0.05$ was taken as significant.

RESULTS

Phytochemical Screening

Phytochemical screening of the *Psidium guajava* aqueous leaf extract shows the presence of the following constituents, tannins, phenols, triterpenes, flavonoid, essential oils, saponins, carotinoid, lectins, vitamins, fibre, faty acid, gallic acid, glutaminic acid and quacetine (Table 1).

Table 1: Phytochemical screening of aqueous leaf extract of *Psidium guajava* (guava).

Phytochemical constituent	leaf
Tannins	++
Phenol	++
Triterpenes	++
Flavonoid	+++
Saponins	+++
Carotenoid	++
Vitamins	+++
Polyphenol	+
Ascorbic acid	++
Aspartic acid	+
Glutaminic acid	+

Key: + = present, ++ = moderately present, +++ = abundantly present

Acute toxicity (LD_{50}) study

No mortality was recorded at the entire LD_{50} experimental dose level in both phases. The LD_{50} of *Psidium guajava* (guava) is estimated to be more than 4000mg/kg body weight as shown in Table 2.

Table 2: Acute toxicity (LD_{50}) study.

Phase one			
Group	Dose (mg/kg)	Death	% mortality
N=3	Body weight	N	N
1	10	0/3	0
2	100	0/3	0
3	1000	0/3	0
Phase two			

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Group N=3	Dose mg/kg Body weight	Death N	% mortality N
1	4000	0/1	0
2	2000	0/1	0
3	1000	0/1	0
4	500	0/1	0

N=Number of mice per group.

The aqueous leaf extract of *Psidium guajava* (guava) exhibit suppressive effect against hyperglycemia (Diabetes mellitus) as shown in the table above. The table shows that the alloxan induced diabetic mice shows a highly significant ($p<0.05$) reduction in blood glucose level (358.00 mg/dl) in the treatment group administered with 450mg/kg of aqueous leaf extract of *psidium guajava* in alloxan induced diabetic mice, the glucose level decreases significantly in standard control group (444.60 mg/dl) contrasted to the diabetic control group (564.00 mg/dl). The aqueous leaf extract 450mg/kg exhibited a highly significant reduction towards the normal control, while the aqueous leaf extract of 150mg/kg shows a minimal reduction (501.80mg/dl) towards the normal control after the first 14 days of administration with aqueous leaf extract of *psidium guajava* as seen in Table 3.

Table 3: Ameliorating effect of 14-day treatment of aqueous leaf extract of *Psidium guajava* on fasting blood glucose level in mice.

Groups	FBG (mg/dl)
Normal control (0.5ml)	102.00±3.74
Diabetic control (0.5ml)	564.00±14.00
Standard drug (5 mg/kg)	444.60±32.02
Extract 150 mg/kg	501.80±10.01
Extract 300 mg/kg	408.20±40.90
Extract 450 mg/kg	358.00±4.96

Value expressed as mean ±SEM, N=5 per group. ($P<0.05$) Significance.

The aqueous leaf extract of *Psidium guajava* (guava) exhibit suppressive effect against hyperglycemia (Diabetes mellitus) as shown in the table above. The table shows that the alloxan induced diabetic mice exhibited a highly significant ($p<0.05$) reduction in blood glucose level (346.80 mg/dl) in the treatment group administered with 450mg/kg of aqueous leaf extract of *psidium guajava* in alloxan induced diabetic mice, the glucose level decreases significantly in standard control group (363.60 mg/dl) contrasted to the diabetic control group (580.40 mg/dl). The aqueous leaf extract 450mg/kg (346.80 mg/dl) and 300mg/kg (356.40 mg/dl) exhibited a highly significant reduction towards the normal control, while the aqueous leaf extract of 150mg/kg shows a minimal reduction (519.60mg/dl) towards the normal control after the second 14 days (28 days) of treatment with aqueous leaf extract of *psidium guajava* (Table 4).

Table 4: Ameliorating effect of 28-day treatment of aqueous leaf extract of *Psidium guajava* on fasting blood glucose level in mice.

Groups	FBG (mg/dl)
Normal control (0.5ml	96.00±2.456
Diabetic control (0.5ml)	580.40±5.80
Standard drug (50 mg/kg)	363.60±24.23
Extract 150 mg/kg	519.60±5.88
Extract 300 mg/kg	356.40±37.1
Extract 450 mg/kg	346.80±2.06

Value expressed as mean ±SEM, N=5 per group. ($P<0.05$) Significance.

Figure 1 and 2 illustrate the effects of a 28-day treatment with *Psidium guajava* aqueous leaf extract on serum ALT and AST activities in alloxan-induced diabetic mice. Untreated diabetic animals showed significantly elevated ALT and AST levels ($p < 0.05$) compared to other groups. In contrast, treatment with *Psidium guajava* extract significantly reduced ALT and AST activities, nearing levels observed in healthy controls.

DOI: 10.56892/bima.v8i3B.848

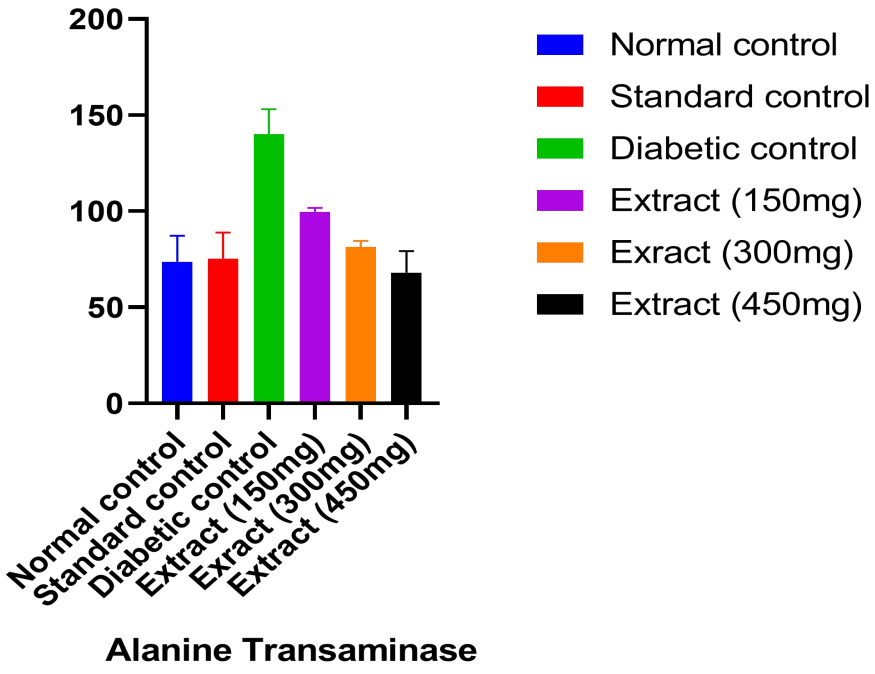

Figure 1: Effect of aqueous leaf extract of *Psidium guajava* (guava) on alanine transaminase in alloxan induced diabetic mice.

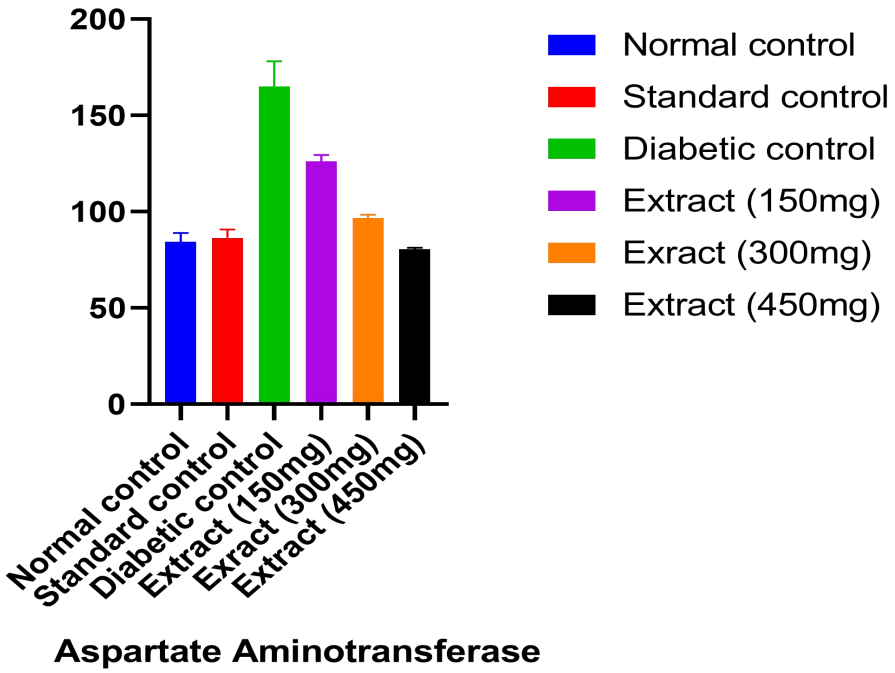

Figure 2: Effect of aqueous leaf extract of *Psidium guajava* (guava) on aspartate aminotransferase in alloxan induced diabetic mice.

DOI: 10.56892/bima.v8i3B.848

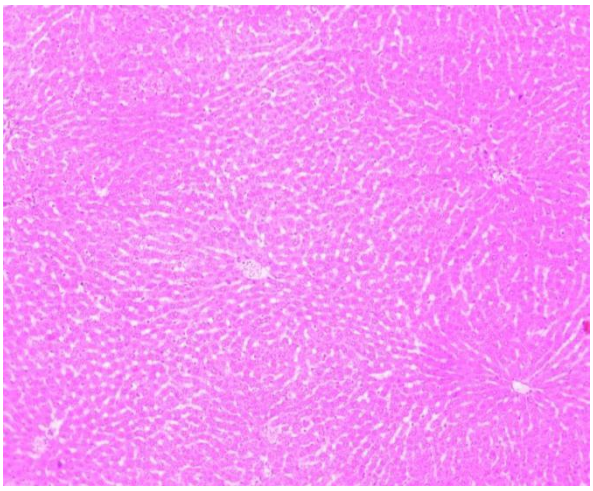

Figure 3: Normal Control.

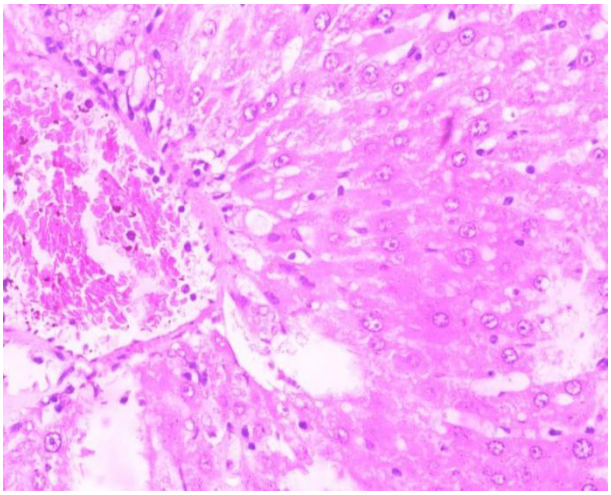

Figure 4: Diabetic control.

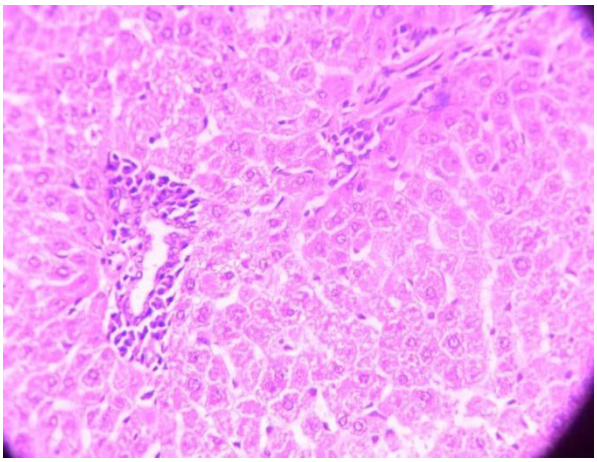

Figure 5: Standard control.

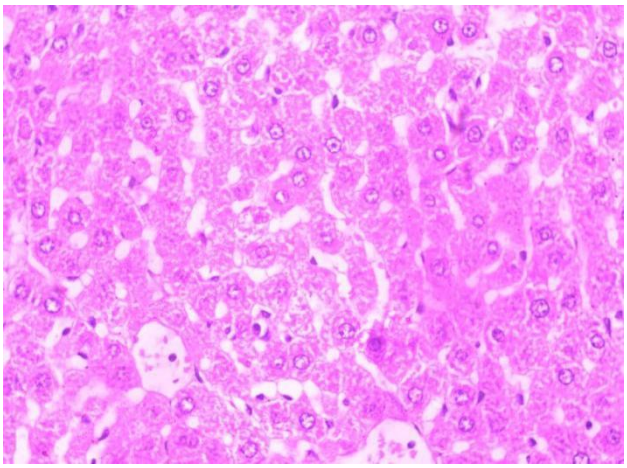

Figure 6: Extract 150mg/kg.

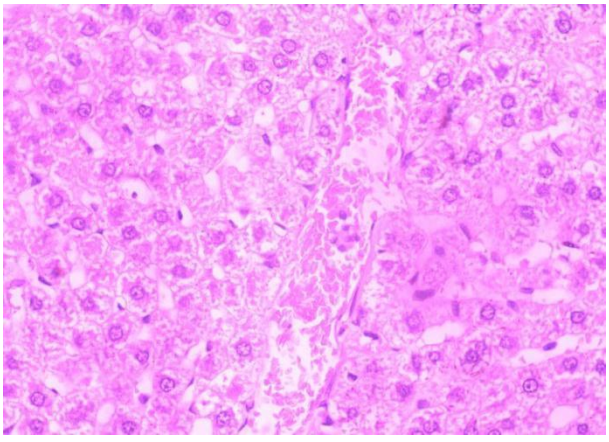

Figure 7: Extract 300mg/kg.

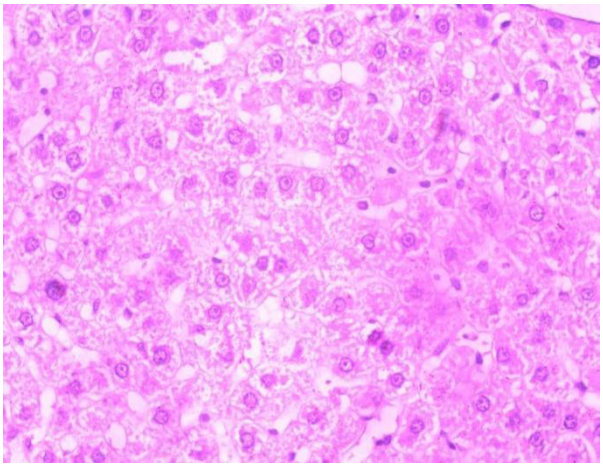

Figure 8: Extract 450mg/kg.

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Histopathological Examination of Diabetic Liver Cells in Mice Treated with Aqueous Leaf Extract of *Psidium guajava*

The hepato-histo-architecture of the liver of mice in normal control group with clear liver lobule structure, well-organized hepatocytes, visible sinusoids, and central veins. The liver parenchyma also appears to be normal with normal kupffer cell distribution (Fig 3). The liver histology of untreated alloxan-induced diabetic mice (Fig. 4) showed pronounced hepatic damage, characterized by necrosis, sinusoidal dilatation, hepatocyte injury, Kupffer cell activation, inflammatory responses, fibrotic changes, and cytoplasmic vacuolation of hepatocytes. Histological examination of the liver in diabetic mice treated with metformin revealed significantly reduced damage, featuring mild degeneration, moderate hepatic sinusoid dilation, and a slight increase in Kupffer cells, accompanied by minimal inflammatory and steatotic changes (Fig. 5).

The hepato-histo-architecture of the liver in alloxan-induced diabetic mice treated with 150mg/kg body weight (b,w) of aqueous leaf extract of *psidium guajava* showing a more uniform and organized hepatocytes, reduced inflammation, less congested sinusoids and less pronounced vacuolization of hepatocyte (Fig. 6). The histo-architecture of the liver in alloxan-induced diabetic mice treated with 300mg/kg body weight (b,w) of aqueous leaf extract of *psidium guajava* showing signs of normal hepatocyte architecture, reduced inflammation, normal sinusoids without marked dilatation or congestion and absence of significant necrosis Fig. 7). The histopathology of the liver in alloxan-induced diabetic mice treated with 450mg/kg body weight (b,w) of aqueous leaf extract of *Psidium guajava* showing regular round hepatocytes, reduced inflammation, hepatocytes with insignificant vacuolization, central veins and sinusoids appear normal without significant congestion or dilation, and some clear areas within the cytoplasm of hepatocytes which could indicate altered glycogen storage which is common in diabetes (Fig. 8).

DISCUSSION

Diabetes mellitus (DM) is one of the most prevailing metabolic disorders, characterized by high level of blood glucose level, which may result from either the pancreas's failure to secrete insulin or the inability of insulin receptors to respond to insulin (Eze and Maxwell, 2021). This condition arises from impaired carbohydrate metabolism, often linked to decreased insulin levels or failure of target organs to respond to insulin (Kumar et al., 2020). It is regarded as a manageable but not curable metabolic disorder that affects approximately 2.8% of the world population (Armocida et al., 2024). Despite significant advancements in diabetes treatment through glucose lowering agents, the ongoing efforts focus discovering innovative treatments to overcome the shortcomings of conventional synthetic medications (Banerjee et al., 2020).

The distinctive capacity of alloxan to selectively destroy pancreatic beta cells was initially detailed by Bingham et al. (2021). Alloxan is a chemical diabetogen commonly employed to initiate hyperglycemic conditions in experimental animals. This glucose analog exhibits selective toxicity towards pancreatic beta cells, primarily due to its intracellular bioaccumulation (Malaisse, 2020). The presence of cysteine's sulfhydryl groups enhances alloxan's inhibitory effects through disulfide bond-mediated enzyme inactivation. In the presence of glutathione, alloxan is reduced to dialuric acid through the alloxan radical. The reduction-oxidation cycle between alloxan and dialuric acid generates reactive oxygen species (ROS) and superoxide radicals through continuous electron transfer.

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Studies have shown that ROS play a crucial role in mediating the cytotoxic effects of alloxan (de Souza Abboud et al., 2020). These ROS induce DNA fragmentation in pancreatic islets, particularly affecting beta cells that are exposed to alloxan monohydrate (Rao and Subrahmanyam, 2017).

Preliminary acute toxicity studies (LD50) of the aqueous leaf extract of *Psidium guajava* were conducted in various phases to determine the lethal oral dosage. Research indicates that 4000 mg or more of aqueous leaf extract can be safely ingested as a decoction. Administration of *Psidium guajava* aqueous leaf extract to alloxan-induced diabetic mice for 28 days resulted in pronounced and statistically significant improvements in chronic hyperglycemia. The notable reduction in blood glucose in these diabetic mice following treatment with the extract may be in conjunction with the occurence of various bioactive compounds, including alkaloids, tannins, flavonoids, triterpenoids, and other chemical constituents, which could contribute to its hypoglycemic effects. Previous research has indicated that these compounds found in *Psidium guajava* leaves are linked to glucose lowering activity (Kumar et al., 2021).

Furthermore, the anti-hyperglycemic effect of the aqueous leaf extract may be linked with its high quercetin content, a flavonoid known to enhance carbohydrate assimilation in liver cells and lower high blood glucose levels in diabetes (Chu et al., 2022). This aligns with findings by Díaz-de-Cerio et al. (2023), which suggest that the substantial quercetin content in *Psidium guajava* leaf extract may offer therapeutic benefits for diabetes management. The significant reduction in hyperglycemia observed in alloxan-induced diabetic mice indicates that the biochemical pathway of *Psidium guajava* is likely not related to insulin secretion, as alloxan-induced diabetes leads to the destruction of β-cells and impaired renal function. This implies that *Psidium guajava* may exert its hypoglycemic effect through a different mechanism of action, possibly involving alternative pathways for glucose utilization and/or insulin sensitivity enhancement. Therefore, the extract may play a role in managing non-insulin dependent diabetes mellitus. The hypoglycemic effect observed with the aqueous leaf extract of *Psidium guajava* in alloxan-induced diabetic mice suggests that this effect may be linked to increased peripheral glucose utilization (Eze and Maxwell, 2021). The results indicated a highly significant ($P<0.001$) anti-hyperglycemic effect, as evidenced by the reduction of high fasting blood glucose levels in the test groups treated with 300 mg/kg and 450 mg/kg doses of the *Psidium guajava* leaf extract compared to both negative and positive controls.

In our study, we have discovered that aqueous leaf extract of *Psidium guajava* significantly lowered high blood sugar levels in T2DM mice in a graded response and this is consistent with the findings of Chu et al., (2022). Additionally, aqueous leaf extract of *Psidium guajava* may alleviate glucose homeostasis and insulin sensitivity in hyperglycemic mice (Chu et al., 2022).

The liver histo-architecture of the mice in the diabetic control group showed significant disorganization, with numerous necrotic hepatocytes and notable structural changes. These alterations were attributed to increased free radical production, leading to heightened mitochondrial oxidative stress, characterized by tissue reaction and cell death, as reported by Yusuf et al. (2024). In contrast, the treatment and standard control groups exhibited only mild to minimal disorganization in their liver histo-architecture, with both healthy and necrotic hepatocytes present. This observation is consistent with the findings of Zhu et al. (2020), which

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highlighted the hepatoprotective effects of the aqueous leaf extract of *Psidium guajava*. The phytochemical analysis from this study provide evidence that presence of flavonoids, known for their antioxidant properties, may be obliged for the extract's ability to enhance hepatic cellular activity (Zhu et al., 2020).

Liver function tests are frequently used to screen for liver diseases, track the progression of existing conditions, and assess the impact of drugs that may be harmful to the liver. Beyond diagnosing and monitoring liver diseases, these tests are also used to determine whether a medicinal plant has hepatotoxic or hepatoprotective properties. In diabetic conditions, serum enzyme activities may increase, indicating tissue damage or reduced clearance (Joshua et al., 2022). However, this elevation can be mitigated by treating diabetes with aqueous leaf extracts of *Psidium guajava*, as demonstrated by a previous study (Ogueri et al., 2014) on alloxan-induced diabetic rats. In our current research, doses of 300 mg/kg and 450 mg/kg of *Psidium guajava* leaf extract significantly lowered aspartate transaminase (AST) and alanine transaminase (ALT) levels, aligning with findings by Roy et al. (2006). In cases of long-term hepatic scarrimg initiated by carbon tetrachloride (CCl4), the higher dose of *Psidium guajava* extract proved more therapeutic than the lower dose, consistent with Roy et al.'s previous report on its hepatoprotective properties. A study by Tella et al. (2019) aligns with our findings. It was observed that AST activity was lowest in the diabetic control group, but treatment with *Psidium guajava* elevated the enzyme activity to concentrations higher than those in untreated and treated normal animals.

The current research revealed a significant ($P<0.05$) change in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the experimental groups contrasred to the untreated diabetic control group, following the administration *Psidium guajava* aqueous leaf extract in alloxan-induced diabetic mice. This finding may be attributed to the occurence of flavonoids in the extract. Research has demonstrated that flavonoids possess a range of protective properties, including anti-allergic, anti-inflammatory, and antioxidant effects, as well as the ability to inhibit platelet aggregation, combat microbial infections, prevent ulcers, safeguard against hepatotoxicity, exhibit antiviral activity, and suppress tumor growth (Kumar et al., 2021).

CONCLUSION

In conclusion, this study revealed that *Psidium guajava* aqueous leaf extract may possess a highly significant ameliorating effect in lowering blood glucose level, hepatoprotevtive potential and mitigating the detrimental effect of hyperglycemia on the liver of male Swiss albino mice.

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