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ANTI-DIABETIC AND SAFETY EVALUATION OF METHANOLIC LEAF EXTRACT OF *PSIDIUM GUAJAVA* IN ALLOXAN-INDUCED DIABETIC WISTAR RATS: A FOCUS ON IL-6 CYTOKINE

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, insulin resistance, and inflammation, mediated by cytokines like interleukin-6 (IL-6). *Psidium guajava* (guava) is widely used in African traditional medicine for diabetes management, though its immunomodulatory effects remain poorly defined.
Aim: This study To evaluate the anti-diabetic, immunomodulatory, and safety profiles of methanolic *Psidium guajava* leaf extract in alloxan-induced diabetic Wistar rats, focusing on IL-6 modulation.
Methods: Thirty Wistar rats were randomized into six groups (n = 5): negative control, diabetic control, glibenclamide-treated, and three extract-treated groups (250, 500, 1000 mg/kg). Diabetes was induced with alloxan (160 mg/kg). Glucose, IL-6, liver and kidney function markers were assessed after 14 days. Phytochemical screening and acute toxicity tests were performed. ELISA kits with known sensitivity and specificity were used for cytokine quantification.
Results: Glucose levels decreased dose-dependently, with the 1000 mg/kg.bwt extract showing a significant reduction ($p < 0.05$), though not surpassing glibenclamide. IL-6 levels varied among groups but showed no statistically significant change ($p > 0.05$). Liver enzymes (AST, ALT) increased significantly in the 250 mg/kg.bwt group, while other markers remained within normal limits. The extract was well-tolerated with an LD50 > 5000 mg/kg.
Conclusion: *Psidium guajava* leaf extract demonstrated anti-diabetic effects and short-term safety but did not significantly modulate IL-6 levels, possibly due to the short treatment duration and the diabetes model used. Further studies using chronic models and longer exposure are recommended.
Keywords: Psidium guajava, IL-6, diabetes mellitus, cytokine, phytochemicals, Wistar rats

INTRODUCTION

Diabetes mellitus (DM) poses a significant global health challenge, characterized by metabolic disturbances and persistent hyperglycemia. It results from either deficient insulin production by pancreatic β-cells (type 1 diabetes mellitus, T1D) or reduced insulin action and secretion (type 2 diabetes mellitus, T2D) [1]. The global prevalence of DM is alarmingly high, with over 463 million people currently affected. Projections estimate this number will reach 578 million by 2030 and 700 million by 2045 [2]. In Africa, approximately 19.4 million individuals, or 4.7% of the adult population aged 20–79, are living with diabetes [2]. Several studies have linked poor glucose control in diabetes to inflammatory processes mediated by cytokines such as interleukin-6 (IL-6) [3]. Chronic inflammation associated with IL-6 overexpression may contribute to complications such as atherosclerosis, impaired lung function, and cardiovascular disease. Medicinal plants are defined as species whose parts flowers, leaves, roots, stems, fruits, or seeds are used directly or in preparation to treat diseases [4]. They are widely employed in traditional medicine, particularly in low-resource settings like Nigeria, where over 80% of plant-derived compounds screened have

demonstrated antidiabetic activity [5,6]. The methanolic extract of P. guajava leaves is rich in bioactive flavonoids, including quercetin and catechins, which have been shown to exert potent anti-inflammatory and insulin-sensitizing effects through modulation of key signaling pathways. Specifically, these phytochemicals inhibit the activation of NF-κB and MAPK cascades [7], which are central to the transcriptional regulation of pro-inflammatory cytokines such as IL-6. IL-6 plays a pivotal role in the pathogenesis of insulin resistance by perpetuating a chronic inflammatory state through sustained activation of these same pathways [8]. Theoretically, the flavonoid constituents of P. guajava should attenuate IL-6 expression via two primary mechanisms: (1) direct suppression of NF-κB-mediated IL-6 gene transcription, and (2) interruption of MAPK-dependent post-translational cytokine signaling. This dual inhibition would consequently improve glycemic control by restoring insulin receptor substrate (IRS) phosphorylation and glucose transporter (GLUT4) translocation in peripheral tissues. Furthermore, quercetin and related polyphenols may enhance pancreatic β-cell function by reducing oxidative stress-mediated apoptosis, thereby addressing both the inflammatory and metabolic components of type 2 diabetes mellitus (T2DM). These mechanistic insights underscore the potential of P. guajava as a multifaceted therapeutic agent, though empirical validation of its cytokine-modulating effects requires further investigation under physiologically relevant experimental conditions.

Psidium guajava (guava), a tropical plant widely cultivated for its fruit, belongs to the Myrtaceae family [9]. It is extensively used in traditional medicine in many parts of the world including India, Africa, and Central America. Its leaves and bark have been employed to treat wounds, gastrointestinal infections, vertigo, and diabetes, among other ailments [10-12]. The WHO's Global Report on Traditional and Complementary Medicine [13] highlights the widespread use of herbal therapies in the African region, with over 85% of countries confirming reliance on traditional medicine for managing chronic diseases, including diabetes. *Psidium guajava* is a such plant widely accessible in Africa and used in folk medicine for managing diabetes. This study aimed to evaluate the anti-diabetic and safety profile of *Psidium guajava* methanolic leaf extract in alloxan-induced diabetic rats, with a specific focus on its effect on IL-6, a pro-inflammatory cytokine involved in the pathogenesis of T2D.

METHODS

Plant collection and Identification

Fresh leaves of *Psidium guajava* were collected from Sokoto South, Sokoto State, Nigeria. The plant material was authenticated at the Herbarium Unit, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto (UDUS), and assigned the voucher number PCG/UDUS/MGRT/0004.

Plant Preparation and Extraction

The leaves were washed with running tap water to remove surface contaminants and air-dried at room temperature. The dried leaves were ground using a blender, sieved into a fine powder, and stored in an airtight container. A known weight of the powder was soaked in distilled water in a round-bottom container and left at room temperature for 24 hours to ensure adequate extraction. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated in an incubator set at 60°C until a solid residue formed. This residue was stored in a sterile, screw-capped container and refrigerated at 5°C until use.

Ethical Approval

Ethical approval for the use of laboratory animals was obtained from the Animal Ethics Committee of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, UDUS, under approval number UDUS/FPS/EA/234A. All experimental protocols conformed to international guidelines on animal welfare, as outlined by the Organization for Economic Co-operation and Development (OECD).

Phytochemical Screening

Phytochemical screening of the methanolic leaf extract of *Psidium guajava* was carried out using standard qualitative procedures [14]. The extract tested positive for several classes of bioactive compounds. Alkaloids were identified by the formation of an orange-red precipitate after treatment with Dragendorff's reagent. Flavonoids were confirmed through the alkaline reagent test, where a yellow color that disappeared upon acidification indicated their presence. Saponins were detected by the appearance of persistent froth after vigorous shaking in distilled water. Cardiac glycosides produced a brown ring at the interface in the Keller-Killiani test, while steroids gave a positive result in the Liebermann Burchard test, marked by brown coloration. Carbohydrates were confirmed by both Molisch's test (purple ring formation) and Fehling's test (brick-red precipitate indicating reducing sugars). Phenolic compounds were identified by a color change (red, blue, green, or purple) upon addition of ferric chloride solution. These results confirm the presence of a rich spectrum of phytochemicals in the extract, many of which are known for their antioxidant, anti-inflammatory, and anti-diabetic properties.

Test for Carbohydrates

Two tests were used to detect carbohydrates in the extract. In Molisch's test, the extract was dissolved in water and two drops of Molisch reagent were added, followed by concentrated sulfuric acid along the sides of the test tube. A purple ring at the interface indicated the presence of carbohydrates. In Fehling's test, 2 ml of the extract was mixed with 2 ml each of Fehling's solutions A and B and boiled for 10 minutes in a water bath. The formation of a red precipitate confirmed the presence of reducing sugars.

Test for Phenols

To detect phenols, the extract was dissolved in water and ferric chloride solution was added dropwise. A red, blue, green, or purple coloration indicated the presence of phenolic compounds.

Experimental Animals

Thirty Wistar albino rats (140–160 g, 8–12 weeks old; 15 males and 15 females) were obtained from the Animal House, Faculty of Pharmaceutical Sciences, UDUS. Animals were fed standard pelletized feed and water ad libitum and were housed under standard laboratory conditions. All procedures complied with the ethical guidelines and were approved by the Institutional Animal Ethics Committee of UDUS.

Acute Toxicity Test (LD_{50})

The LD_{50} of the methanolic extract of *Psidium guajava* was determined using Lorke's method [15]. In phase one, nine rats were divided into three groups (n=3), each receiving 10, 100, and 1000 mg/kg.bwt respectively. No mortality was observed after 24 hours. In phase two, three rats were administered 1600, 2900, and 5000 mg/kg. Again, no mortality or behavioral changes were noted, indicating an LD_{50} greater than 5000 mg/kg.

Grouping and Treatment

After acclimatization, the animals were divided into six groups (n=5): Group I – normal control, Group II – diabetic untreated control, Group III – diabetic + glibenclamide (3 mg/kg), Group IV – diabetic + 250 mg/kg.bwt extract, Group V – diabetic + 500 mg/kg.bwt extract, and Group VI – diabetic + 1000 mg/kg.bwt extract.

Induction of Diabetes

Diabetes was induced using intraperitoneal injection of alloxan monohydrate (160 mg/kg). Blood glucose levels were confirmed to be 72 hours post-induction using a glucometer. Alloxan-induced diabetes primarily models Type 1 DM via pancreatic β-cell cytotoxicity. Nonetheless, it produces hyperglycemia and mild insulin resistance sufficient for preliminary screening of anti-diabetic compounds.

Sample Collection and Processing

Blood samples were collected via cardiac puncture after chloroform anesthesia. Approximately 2 ml of blood was drawn into plain tubes, centrifuged at 4000 g for 1 minute, and the serum was stored in cryovials at −20°C for ELISA analysis.

Laboratory Analysis

Glucose, electrolytes, and urea were measured according to manufacturer protocols. IL-6 concentrations were determined using ELISA kits (Nanjing Pars BiochemCo Ltd, PRS-30378Ra).

Determination of IL-6 Concentration

Serum interleukin-6 (IL-6) levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Pars BiochemCo Ltd, PRS-30378Ra, Rat IL-6 ELISA Kit), following the manufacturer's instructions.

Assay-Principle

Serum interleukin-6 (IL-6) levels were measured using a commercially available sandwich ELISA kit (Nanjing Pars Biochem Co Ltd, PRS-30378Ra, Rat IL-6 ELISA Kit), according to the manufacturer's protocol. The kit has a sensitivity of 1.0 pg/mL, with intra-assay and inter-assay coefficients of variation (CV) below 8% and 10%, respectively. No cross-reactivity with other cytokines was reported. The assay is based on the principle of a double-antibody sandwich technique. Microtiter plate wells were pre-coated with purified anti-rat IL-6 monoclonal antibodies. Standards, blanks, and serum samples (10 µL sample mixed with 40 µL sample diluent) were added to the wells. The plate was incubated at 37°C for 30 minutes, followed by multiple washing steps to remove unbound components. A horseradish peroxidase (HRP)-conjugated secondary antibody was added to form an antibody antigen enzyme complex. After a second incubation and washing, tetramethylbenzidine (TMB) substrate solution was added, and the plate was incubated for 15 minutes at 37°C. The enzymatic reaction was terminated by the addition of a stop solution (sulfuric acid), changing the color from blue to yellow. Absorbance was measured at 450 nm using a microplate reader. The concentration of IL-6 in each sample was determined by comparing the optical density (OD) values to a standard calibration curve generated from known IL-6 concentrations.

Procedure

Briefly, 10 µL of serum was mixed with 40 µL of sample diluent and added to designated wells. Standards were loaded in duplicate, and blank wells were included. The plate was incubated at 37°C for 30 minutes, washed thoroughly, and incubated with 50 µL of HRP-conjugated reagent. After a second incubation and washing cycle, substrate solution (TMB) was added and color development proceeded for 15 minutes at 37°C. The reaction was terminated with 50 µL of stop solution, and the absorbance was read at 450 nm using a microplate reader. All samples were analyzed in accordance with the kit protocol and OD values were referenced against the standard curve for IL-6 quantification.

Statistical Analysis

Data were analyzed using SPSS version 25. Continuous variables were presented as mean ± standard deviation. One-way ANOVA followed by Bonferroni post hoc test was used to determine statistical significance, set at $p < 0.05$.

RESULTS

Table 1: The table of procedure and result of phytochemicals of *Psidium guajava* leaf extracts

Phytochemicals	Test	Observation	Results
Carbohydrate	Molisch's Test	Brown interference	++
	Fehling Test	Dark orange colour	++
Saponin	Frothing Test	Foam remained for more than 30 minutes	++
Phenols	Ferric chloride Test	Blue-Black colour	++
Flavonoids	Ferric chloride Test	Blue-Black colour	++
	Alkaline Test	Colorless	+++
	Shinoda's Test	Orange colour	+
Tannis	Ferric chloride Test	Blue-Black colour	++
	Lead Acetate Test	Precipitation	+
Alkaloids	Mayer's Test	Precipitation	+
	Wagner's Test	Precipitation	+
	Hager's Test	Precipitation	+
Steroids (Terpenoids)	Salkowski's Test	Golden yellow colour	++
	Liebermann-Buchard's Test	Brown interface	+
Anthraquinones Glycoside	Borntrager's Test	No reaction	_
Cardiac Glycosides	Killer-Killiani Test	Brown interface	+++

Keys: (+) indicate presence, (++) indicate significant presence, (-) indicate absence

Acute Oral Toxicity (Median Lethal Dose LD_{50})

Phase I of acute oral toxicity studies

Table 2 depicts the result of phase 1 median lethal dose determination of methanolic leaf extracts of *Psidium guajava* after a single oral dose administration of 10mg/kg.bwt, 100mg/kg.bwt, and 1000/mg/kg.bwt for group 1, group 11 and group 111, respectively. No death or any sign of toxicity was observed in all three groups after 24 hours and up to the end of the study.

Table 2: Results of phase 1 of acute oral toxicity study (Median Lethal Dose, LD_{50} Determination)

Groups	N	Dose (mg/Kg.bwt)	Mortality
Group I	3	10	0
Group II	3	100	0
Group III	3	1000	0

Key: N = number of rats; mg/Kg.bwt = milligrams per kilogram of body weight

Phase II of Oral Toxicity Study

Table 3: Results of Phase 2 Acute Oral Toxicity Study (Median Lethal Dose, LD_{50} Determination)

Groups	N	Dose (mg/Kg.bwt)	Mortality
Group I	1	1900	0
Group II	1	2600	0
Group III	1	5000	0

Key: N = number of rats; mg/Kg.bwt = milligrams per kilogram of body weight

The results of phase 11 of acute oral toxicity study (median lethal dose determination) of the methanolic leaf extracts of *Psidium guajava* after a single oral dose administration of 1900mg/kg.bwt, 2600mg/kg.bwt, 5000mg/kg.bwt for group I, group I and group III respectively are depicted in table 3. No mortality was documented after 24 hours shown in Table 3 below:

Table 4: Results of the glucose level of rats after induction and after treatment.

Groups	WTBI (g)	WTAT (g)	GLAI (mmol/L)	GLAT (mmol/L)
G1	124.00 ± 6.00	144.00 ± 8.00	4.40 ± 1.00	4.10 ± 1.00
G2	129.00 ± 2.00	136.00 ± 4.00	23.40 ± 11.10	21.25 ± 13.25
G3	160.50 ± 8.50	171.00 ± 9.00	31.70 ± 2.80	27.25 ± 2.55
G4	108.00 ± 5.00	126.00 ± 4.00	8.60 ± 1.00	5.35 ± 4.50
G5	145.00 ± 5.00	209.00 ± 8.00	8.85 ± 1.45	6.75 ± 5.50
G6	141.50 ± 21.5	161.50 ± 29.50	21.10 ± 13.40	5.65 ± 7.50

Key:

WTBI: = weight before induction.

WTAT: = weight after induction

GLAI: = glucose level after induction (after 72 hours of induction)

GLAT: = glucose level after treatment (before sacrifice)

Glucose normal range: 4.02mmol/L - 7.28mmol/L

Assessment of Weight in Alloxan-Induced Diabetic Rats, Treated with Methanolic Leaf Extract of *Psidium Guajava*

Table 5 depicts the assessment of the weight of Wistar rats induced with alloxan and treated with the methanolic leaf extract of *Psidium guajava*. The result was expressed as means ± standard error of the mean. For the weight of the rats after induction and treatment (WTBI and WBAT), there is a significant difference in WBAT in the p-value of the groups (p-value <0.05).

Table 5: The WTBI and WTAT Status in the study groups.

Groups	N	WTBI (g)	WTAT (g)
G1	5	124.00 ± 6.00	144.00 ± 8.00[a]
G2	5	129.00 ± 2.00	136.00 ± 4.00[ab]
G3	5	160.50 ± 8.50	171.00 ± 9.00[a]
G4	5	108.00 ± 5.00	126.00 ± 4.00[a]
G5	5	145.00 ± 5.00	209.00 ± 8.00[c]
G6	5	141.50 ± 21.5	161.50 ± 29.50[a]
F-value		3.224	4.884
P-value		0.450	0.015

Values are expressed as mean ± SEM, N= number of Wistar rat per group, Group 1=Negative control, Group 2= Positive control (Alloxan 160mg/kg.bwt only), Group 3=Glibenclamide group (Alloxan 160mg/kg.bwt only), Group 4= Alloxan (160mg/kg.bwt)+ Extract (250mg/kg.bwt), Group 5= Alloxan (160mg/kg.bwt) + Extract (500mg/kg.bwt), Group 6= Alloxan (160mg/kg.bwt) + Extract (1000mg/kg.bwt), p-value <0.05 =significant, and >0.05 =not significant, values with the same superscript have no significant differences.

Graphical Representation of Weight Before Induction and Weight After Treatment

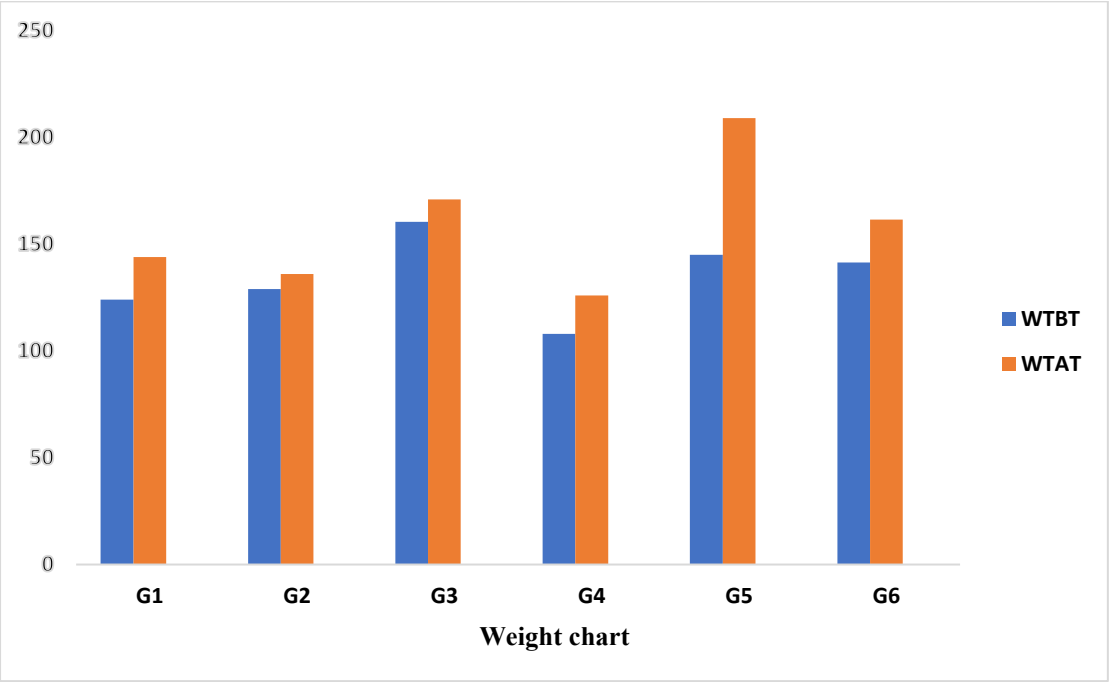


Figure 1: The bar chat of weight expression by research groups.

Key

WTBI: weight before induction

WTAT: weight after induction

Group 1: negative control

Group 2: positive control

Group 3: glibenclamide control

Group 4: 250mg/kg.bwt of methanolic leaf extract of *Psidium guajava*

Group 5: 500mg/kg.bwt of methanolic leaf extract of *Psidium guajava*

Group 6: 1000mg/kg.bwt of methanolic leaf extract of *Psidium guajava*

Table 6: Shows results for the effect of methanolic leaf extract of *Psidium guajava* on the glucose level of alloxan induced diabetic Wistar rats.

Groups	N	Dose	GLAI (mmol/L)	GLAT (mmol/L)
G1	5	Negative Control	4.40 ± 1.00	4.10 ± 1.00
G2	5	Positive Control	23.40 ± 11.10	21.25 ± 13.25
G3	5	Glibenclamide Treatment	31.70 ± 2.80	27.25 ± 2.55
G4	5	250mg/kg.bwt extract	8.60 ± 1.00	5.35 ± 4.50
G5	5	500mg/kg.bwt extract	8.85 ± 1.45	6.75 ± 5.50
G6	5	1000mg/kg.bwt extract	21.10 ± 13.40	5.65 ± 7.50
F-value			2.176	3.205
P-value			0.370	0.260

Values are expressed as mean ± SEM, N= number of Wistar rat per group, Group 1=Negative control, Group 2= Positive control (Alloxan 160mg/kg.bwt only), Group 3=Glibenclamide group (Alloxan 160mg/kg.bwt only), Group 4= Alloxan (160mg/kg.bwt)+ Extract (250mg/kg.bwt), Group 5= Alloxan (160mg/kg.bwt) + Extract (500mg/kg.bwt), Group 6= Alloxan (160mg/kg.bwt) + Extract (1000mg/kg.bwt), p-value <0.05 =significant, and >0.05 =not significant, values with the same superscript have no significant differences.

Graphical Representation of Glucose Level After Induction and Glucose Level After Treatment

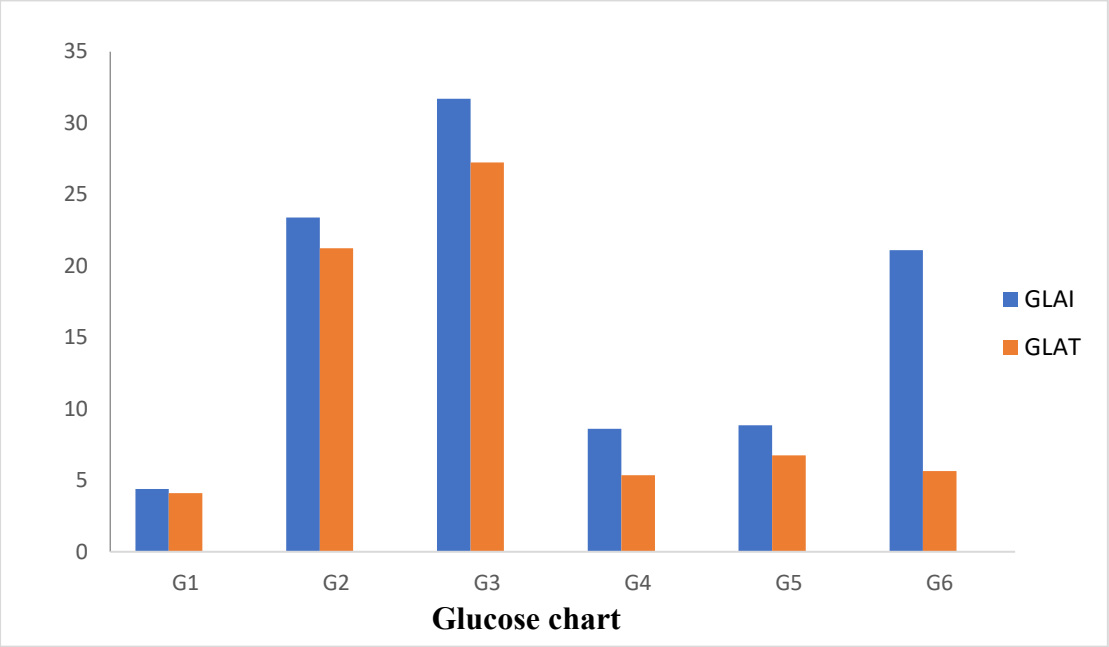


Figure 2: The bar chart of glucose expression by research group

Key

BT: Before treatment

AT: After treatment

Group 1: negative control

Group 2: positive control

Group 3: glibenclamide control

Group 4: 250mg/kg.bwt of methanolic leaf extract of *Psidium guajava*

Group 5: 500mg/kg.bwt of methanolic leaf extract of *Psidium guajava*

Group 6: 1000mg/kg.bwt of methanolic leaf extract of *Psidium guajava*

Table 7a: Effect of Methanolic leaf extract of *Psidium guajava* on (Kidney Function Test) KFT- in different groups of alloxan induced diabetes in male and female Wistar rat.

Group	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
G1	141.00	4.250	102.00	24.00	6.050	0.750
G2	141.50	4.250	98.00	24.00	5.150	0.850
G3	141.50	4.550	96.50	23.50	6.350	0.850
G4	140.00	4.550	89.50	21.50	7.500	0.900
G5	138.00	4.600	92.50	22.00	8.150	0.950
G6	138.50	4.300	95.17	25.00	6.250	0.850

Values are expressed as mean ± SEM, N= number of Wistar rat per group, Group 1=Negative control, Group 2= Positive control (Alloxan 160mg/kg.bwt only), Group 3=Glibenclamide group (Alloxan 160mg/kg.bwt only), Group 4= Alloxan (160mg/kg.bwt)+ Extract (250mg/kg.bwt), Group 5= Alloxan (160mg/kg.bwt) + Extract (500mg/kg.bwt), Group 6= Alloxan (160mg/kg.bwt) + Extract (1000mg/kg.bwt), p-value <0.05 =significant, and >0.05 =not significant, values with the same superscript have no significant differences.

Table 7 depicts the assessment of kidney function test of Wistar rats induced with alloxan and treated with methanolic leaf extract of *Psidium guajava*. The result was expressed as means ± standard error of mean. For the kidney function test of the rats after induction and treatment (Na, K, Cl, HCO_3, Urea, Creat), there is no significant difference in the p-value of the groups (p-value >0.05).

Table 7b: Effect of Methanolic leaf extract of *Psidium guajava* on (Kidney Function Test) KFT- Concentration in alloxan induced diabetes in male and female Wistar rat.

Groups	N	Dose	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
G1	5	Negative Control	141.00 ± 1.00	4.250 ± 0.05	102.00 ± 1.00
G2	5	Positive Control	141.50 ± 1.50	4.250 ± 0.25	98.00 ± 7.00
G3	5	GlibenclamideTreatment	141.50 ± 0.50	4.550 ± 0.25	96.50 ± 1.50
G4	5	250mg/kg.bwt extract/14 days	140.00 ± 2.00	4.550 ± 0.25	89.50 ± 0.50
G5	5	500mg/kg.bwt extract /14days	138.00 ± 1.00	4.600 ± 0.00	92.50 ± 0.50
G6	5	1000mg/kg.bwt extract /14 days	138.50 ± 0.50	4.300 ± 0.20	95.17 ± 1.50
F-value			1.606	0.722	2.244
P-value			0.860	1.000	0.680

Table 7c Effect of Methanolic leaf extract of *Psidium guajava* on (Kidney Function Test) in alloxan-induced diabetes in male and female Wistar rat.

Groups	N	Dose	HCO_3^- (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
G1	5	Negative Control	24.00 ± 2.00	6.050 ± 1.35	0.750 ± 0.05
G2	5	Positive Control	24.00 ± 0.50	5.150 ± 0.05	0.850 ± 0.15
G3	5	Glibenclamide Treatment	23.50 ± 0.50	6.350 ± 0.55	0.850 ± 0.05
G4	5	250mg/kg.bwt extract/14 days	21.50 ± 1.50	7.500 ± 0.30	0.900 ± 0.05
G5	5	500mg/kg.bwt extract /14days	22.00 ± 0.00	8.150 ± 0.150	0.950 ± 0.00
G6	5	1000mg/kg.bwt extract /14 days	25.00 ± 1.00	6.250 ± 0.75	0.850 ± 0.05
F-value			1.503	2.483	8.150
P-value			1.710	0.730	0.374

Values are expressed as mean ± SEM, N= number of Wistar rat per group, Group 1=Negative control, Group 2= Positive control (Alloxan 160mg/kg.bwt only), Group 3=Glibenclamide group (Alloxan 160mg/kg.bwt only), Group 4= Alloxan (160mg/kg.bwt)+ Extract (250mg/kg.bwt), Group 5= Alloxan (160mg/kg.bwt) + Extract (500mg/kg.bwt), Group 6= Alloxan (160mg/kg.bwt) + Extract (1000mg/kg.bwt), p-value <0.05 =significant, and >0.05 =not significant, values with the same superscript have no significant differences.

Estimation of Liver Function Tests in Alloxan-Induced Diabetic Rats, Treated with Methanolic Leaf Extract of *Psidium Guajava* Using Anova

Table 8: Effect of Methanolic leaf extract of *Psidium guajava* on Liver Function Test in alloxan-induced diabetes in male and female Wistar rats.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TP (g/dL)	ALB (g/dL)	DB (mg/dL)	TB (mg/dL)
G1	7.L50	9.50	77.00	68.50	38.50	0.250	0.650
G2	9.00	11.00	83.00	66.00	38.00	0.200	0.700
G3	8.50	10.50	80.50	67.50	37.00	0.250	0.750
G4	12.50	14.50	90.50	68.50	37.00	0.250	0.950
G5	6.00	9.00	85.50	68.00	38.50	0.250	0.850
G6	6.00	10.50	77.50	66.00	37.83	0.250	0.800

Values are expressed as mean ± SEM, N= number of Wistar rat per group, Group 1=Negative control, Group 2= Positive control (Alloxan 160mg/kg.bwt only), Group 3=Glibenclamide group (Alloxan 160mg/kg.bwt only), Group 4= Alloxan (160mg/kg.bwt)+ Extract (250mg/kg.bwt), Group 5= Alloxan (160mg/kg.bwt) + Extract (500mg/kg.bwt), Group 6= Alloxan (160mg/kg.bwt) + Extract (1000mg/kg.bwt), p-value <0.05 =significant, and >0.05 =not significant, values with the same superscript have no significant differences.

Liver function parameters (AST, ALT, ALP, total protein, albumin, total bilirubin, and direct bilirubin) were assessed in alloxan-induced diabetic rats treated with varying doses of *Psidium guajava* methanolic leaf extract. Results are presented as mean ± SEM and analyzed using one-way ANOVA. Significant differences ($p < 0.05$) were observed in AST and ALT levels across groups, while ALP, TP, ALB, TB, and DB remained statistically unchanged ($p > 0.05$). The 250 mg/kg.bwt group exhibited significantly elevated AST and ALT compared to all other groups, suggesting possible hepatic enzyme induction at this dose. Conversely, the 500 mg/kg.bwt group recorded the lowest AST and ALT values, significantly lower than the diabetic control, glibenclamide, and 250 mg/kg.bwt groups. The 1000 mg/kg.bwt dose produced intermediate enzyme levels, not significantly different from the negative control, indicating relative hepatic stability. The glibenclamide group demonstrated enzyme levels moderately higher than the negative control but lower than the 250 mg/kg.bwt group.

Overall, the extract appeared hepatologically safe at 500 and 1000 mg/kg.bwt doses, whereas the 250 mg/kg.bwt dose was associated with mild elevations in transaminases. These findings suggest dose-dependent modulation of hepatic enzymes, with no evidence of extract-induced hepatotoxicity at therapeutic dose.

Table 9: Effect of Methanolic leaf extract of *Psidium guajava* on Liver Function Test (LFT) in alloxan-induced diabetes in male and female Wistar rat.

Groups	Dose	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TP (g/dL)
G1	Negative Control	7.5 ± 0.5[a]	9.50 ± 0.5[a]	77.0 ± 3.0	68.5 ± 0.5
G2	Positive Control	9.0 ± 1.0[ab]	11.0 ± 1.0[ab]	83.0 ± 2.0	66.0 ± 1.0
G3	Glibenclamide treatment	8.5 ± 0.5[a]	10.5 ± 0.5[a]	80.5 ± 0.5	67.5 ± 1.5
G4	250mg/kg.bwt extract	12.5 ± 0.5[c]	14.5 ± 0.5[c]	90.5 ± 1.5	68.5 ± 0.5
G5	500mg/kg.bwt extract	6.0 ± 1.0[a]	9.0 ± 1.0[a]	85.5 ± 8.5	68.0 ± 0.5
G6	1000mg/kg.bwt extract	7.5 ± 1.5[a]	10.5 ± 1.5[a]	77.5 ± 3.5	66.0 ± 1.0
F-value		5.880	4.520	1.588	1.800
P-value		0.021	0.035	0.580	1.000

Table 10: Effect of Methanolic leaf extract of *Psidium guajava* on Liver Function Test (LFT) in alloxan induced diabetes in male and female Wistar rat.

Group	N	Dose	ALB (g/dL)	DB (mg/dL)	TB (mg/dL)
G1	5	Negative Control	38.50 ± 0.50	0.250 ± .050	0.650 ± 0.05
G2	5	Positive Control	38.00 ± 0.00	0.200 ± 0.00	0.700 ± 0.20
G3	5	Glibenclamide Treatment	37.00 ± 0.00	0.250 ± 0.05	0.750 ± 0.05
G4	5	250mg/kg.bwt extract	37.50 ± 0.50	0.250 ± 0.05	0.950 ± 0.05
G5	5	500mg/kg.bwt extract	38.50 ± 0.50	0.250 ± 0.05	0.850 ± 0.05
G6	5	1000mg/kg.bwt extract	37.83 ± 0.50	0.250 ± 0.05	0.800 ± 0.00
F-value			2.200	4.680	1.400
P-value			0.450	0.200	0.590

Values are expressed as mean ± SEM, N= number of Wistar rat per group, Group 1=Negative control, Group 2= Positive control (Alloxan 160mg/kg.bwt only), Group 3=Glibenclamide group (Alloxan 160mg/kg.bwt only), Group 4= Alloxan (160mg/kg.bwt)+ Extract (250mg/kg.bwt), Group 5= Alloxan (160mg/kg.bwt) + Extract (500mg/kg.bwt), Group 6= Alloxan (160mg/kg.bwt) + Extract (1000mg/kg.bwt), p-value <0.05 =significant, and >0.05 =not significant, values with the same superscript have no significant differences.

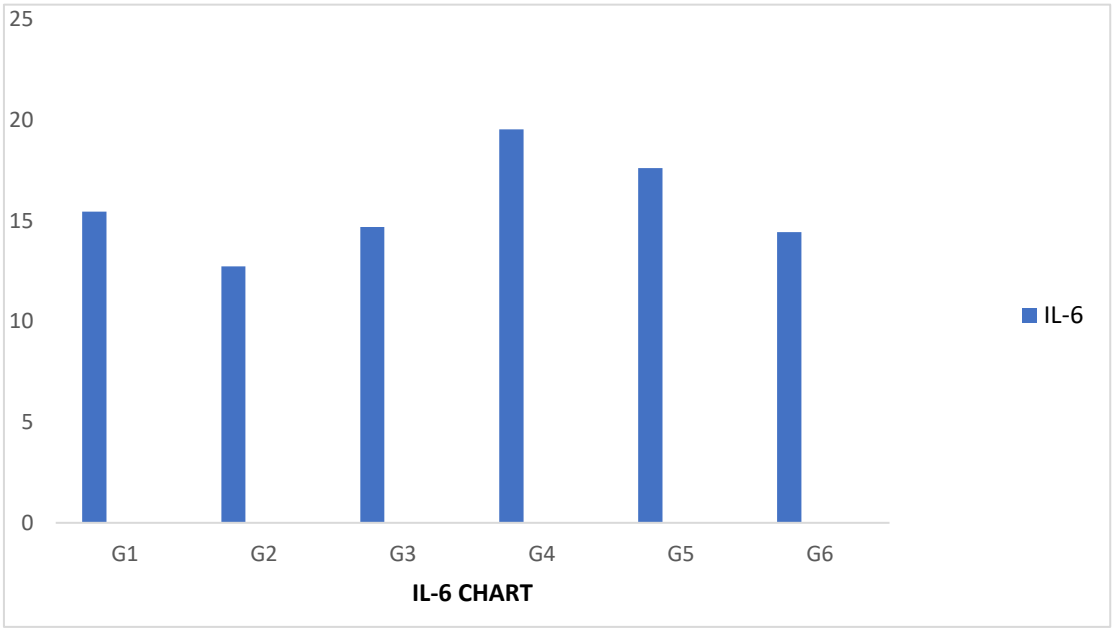
Table 11: Assessment of Rat IL-6 In Alloxan Induced Diabetic Rats, Treated with Methanolic Leaf Extract of *Psidium Guajava*

Table 11 Depicts the assessment of rat IL-6 of Wistar rats induced with alloxan and treated with methanolic leaf extract of *Psidium guajava*. The result was expressed as mean ± standard error of mean. For the KFT and some LFT of the rats after treatment (IL-6), there was no significant differences in the p-value of the groups (p-value >0.05).

Groups	N	Dose	IL-6 (pg/mL)
G1	5	Negative Control	15.450 ± 1.192
G2	5	Positive Control	12.737 ± 2.737
G3	5	Glibenclamide Treatment	14.684 ± 0.256
G4	5	250mg/kg.bwt extract	19.538 ± 0.121
G5	5	500mg/kg.bwt extract	17.616 ± 0.901
G6	5	1000mg/kg.bwt extract	14.428 ± .1.533
F-value			2.950
P-value			0.226

Values are expressed as mean ± SEM, N= number of Wistar rat per group, Group 1=Negative control, Group 2= Positive control (Alloxan 160mg/kg.bwt only), Group 3=Glibenclamide group (Alloxan 160mg/kg.bwt only), Group 4= Alloxan (160mg/kg.bwt)+ Extract (250mg/kg.bwt), Group 5= Alloxan (160mg/kg.bwt) + Extract (500mg/kg.bwt), Group 6= Alloxan (160mg/kg.bwt) + Extract (1000mg/kg.bwt), p-value <0.05 =significant, and >0.05 =not significant, values with the same superscript have no significant differences.

Graphical Representation of Serum IL-6



Key

Group 1: negative control
Group 2: positive control
Group 3: glibenclamide control
Group 4: 250mg/kg.bwt of methanolic leaf extract of *Psidium guajava*
Group 5: 500mg/kg.bwt of methanolic leaf extract of *Psidium guajava*
Group 6: 1000mg/kg.bwt of methanolic leaf extract of *Psidium guajava*
Figure 4.3: The bart of IL-6 expression of research groups

DISCUSSION

The methanolic extract of *Psidium guajava* leaves demonstrated a rich phytochemical profile including flavonoids, alkaloids, cardiac glycosides, saponins, tannins, phenols, and steroids. These compounds are known for their antioxidant, anti-inflammatory, and anti-diabetic properties [16]. The presence of these bioactive compounds in the extract suggests a plausible mechanism for its pharmacological effects, particularly in glycemic regulation. Acute toxicity testing revealed no mortality or observable signs of toxicity even at a high dose of 5000 mg/kg. This finding aligns with previous studies [17] and confirms the safety of *P. guajava* for short-term administration in experimental models.

In this study, *P. guajava* extract exhibited a dose-dependent hypoglycemic effect in alloxan-induced diabetic rats. The 1000 mg/kg.bwt dose achieved the most notable reduction in blood glucose levels, followed by the 500 mg/kg.bwt and 250 mg/kg.bwt groups. Interestingly, these effects were more pronounced than the glibenclamide-treated group, indicating the extract's strong potential as an anti-diabetic agent. These findings support previous work by Tandi *et al.* [18], who also reported glucose-lowering effects of *P. guajava* in diabetic rats. Although the ANOVA test revealed no statistically significant difference across groups ($p > 0.05$), the trend and mean

differences strongly support a biologically relevant glucose-lowering effect. The apparent lack of statistical significance may stem from inter-group variability or small sample size.

Renal function markers including Na^+, K^+, Cl^-, HCO_3^-, urea, and creatinine were within normal ranges across all experimental groups. No statistically significant differences ($p > 0.05$) were observed. This suggests that alloxan-induced diabetes in this study did not compromise renal function, and that *P. guajava* extract did not exert nephrotoxic effects. These findings are consistent with established reference ranges for rat renal physiology and support the renal safety of the extract. The preservation of renal function further enhances the therapeutic appeal of *P. guajava* in diabetic management.

Liver function tests revealed minor alterations. While total protein, albumin, bilirubin, and ALP levels remained stable across groups, significant differences ($p < 0.05$) were observed in AST and ALT levels, particularly in the group treated with 250 mg/kg.bwt extract. This elevation may suggest transient hepatic stress or enzyme modulation by constituents of the extract. These results align with Friday *et al.* [19], who observed similar non-significant variations in hepatic enzymes following *P. guajava* administration. The findings are also partially consistent with Qian and Nihorimbere [20], who noted potential hepatoprotective and enzyme-modulating effects of *P. guajava* due to its polyphenolic content.

Contrary to expectations, IL-6 levels did not differ significantly between groups ($p > 0.05$). Although the extract-treated groups, particularly those receiving 250 mg/kg.bwt and 500 mg/kg.bwt showed numerically elevated IL-6 concentrations relative to diabetic control, these changes lacked statistical significance. Interestingly, the diabetic control group recorded a lower mean IL-6 level than the negative control, an observation that further supports the hypothesis of a delayed or variable cytokine response under the study's conditions. Several plausible factors may explain these results. First, the duration of the experiment (14 days) may have been too short to fully capture the dynamic changes in IL-6 expression, which are known to vary over time following diabetic induction. Literature reports indicate that IL-6 levels in diabetic models often show a biphasic or delayed elevation, especially in models of insulin resistance [21,8]. For instance, in streptozotocin (STZ)- or high-fat diet-induced models, IL-6 elevation becomes more prominent after several weeks, reflecting sustained low-grade inflammation typically associated with Type 2 diabetes mellitus (T2DM). In contrast, the alloxan model, which predominantly causes β-cell cytotoxicity, may elicit a more acute and transient cytokine response, potentially underestimating chronic inflammatory markers such as IL-6. Furthermore, IL-6 secretion is highly context-dependent and influenced by factors such as time post-induction, tissue specificity, and the interaction with other cytokines like TNF-α and IL-1β [22,23]. Since only IL-6 was assayed in this study, it is possible that relevant changes in systemic inflammation were mediated by other cytokines, which were not captured. Several studies have highlighted that TNF-α often precedes IL-6 in inflammatory cascades, particularly in metabolic syndrome and T2DM models [24,25].

Another important consideration is that some phytochemicals present in *Psidium guajava*, such as quercetin and ellagic acid, may exert both immunosuppressive and immunostimulatory effects depending on dose and exposure duration [16]. These dual effects may partially explain the non-linear trend in IL-6 levels across the treatment groups. Notably, previous studies by Qian and Nihorimbere [20)] and Sharma *et al.* [23] suggest that guava extract can modulate cytokine expression via NF-κB and MAPK pathway inhibition, but these pathways may require longer exposure or synergistic activation by metabolic stressors to show measurable downstream effects.

Given these variables, the current study's IL-6 findings, though inconclusive, do not undermine the potential immunomodulatory effect of *P. guajava*. Instead, they highlight the need for longitudinal studies assessing IL-6 and a broader panel of inflammatory markers over time. Future experiments should consider extending treatment duration (4–8 weeks), employing T2DM-

specific models (e.g., high-fat diet + STZ), and incorporating histopathological and tissue-specific cytokine analyses to better characterize the immune-regulatory potential of *P. guajava* extract in metabolic disorders.

CONCLUSION:

Based on the findings of this study, the phytochemicals detected in methanolic leaf extract of Psidium guajava includes steroids, cardiac glycosides, carbohydrates, saponins, phenols, flavonoids, tannins and alkaloids, The acute toxicity study LD50 of aqueous leaf extract of Psidium guajava is proven to be safe at dose greater than 5000 mg/kg. Glucose level shows no significant ($P>0.05$), However, it was also found that the glucose level decreased after treatment of 1000mg/kg *Psidium guajava* extract administered to Wistar rat compared to the glucose level after inducing diabetes, the use of the extract is dose dependant as higher dose is more effective. The Na, K,Cl, HCO_3,Urea and Creatinine showed no significant differences across all the groups for kidney function while ALT and AST showed significant differences in some of the groups among the liver function test performed, The serum IL-6 cytokine shows there is no significant differences across all the groups.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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