RESEARCH ARTICLE

Phytochemical and Pharmacological Investigation on *Mollugo Nudicaulis* Lam for Anti-Diabetic Activity

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Received: 23th Aug, 2024; Revised: 10th Oct, 2024; Accepted: 30th Oct, 2024; Available Online: 25th Dec, 2024

ABSTRACT
This study explores the anti-diabetic potential of *Glinus lotoides* and *Mollugo cerviana* from the *Molluginaceae* family. *Glinus lotoides* is traditionally used in Ethiopian medicine, while *Mollugo cerviana* is rich in flavonoids, particularly apigenins, known for their anti-diabetic properties. The research involves extract preparation, *in vitro* screening, phytochemical analysis, and bioactive compound isolation. *In vivo* and *in silico* studies, including molecular docking, will evaluate their mechanisms of action. This study aims to validate traditional claims and identify potential plant-based anti-diabetic agents.
Keywords: Anti-diabetic, *Glinus lotoides*, *Mollugo cerviana*, *Molluginaceae*, flavonoids, apigenins, phytochemical analysis, *in vitro*, *in vivo*, *in silico*, molecular docking.
How to cite this article: Gurwinder Pal Singh, Sushil Bhargav. Phytochemical and Pharmacological Investigation on *Mollugo Nudicaulis* Lam for Anti-Diabetic Activity. International Journal of Pharmaceutical Quality Assurance. 2024;15(4):2898-2916. doi: 10.25258/ijpqa.15.4.103
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Diabetes is a manageable disease, as noted by Clayton M. Christensen, but remains a major global health concern. Historical records date back to ancient Egypt and India, where it was termed *madhumeha* or "honey pee." Despite advancements from insulin therapy to modern treatments, diabetes remains a top cause of death, affecting over 422 million people worldwide, with cases projected to reach 700 million by 2045. India is expected to see the largest increase due to rising obesity and lifestyle changes[1-5].

Types of Diabetes

Type 1 Diabetes – An autoimmune condition causing insulin deficiency, often appearing in childhood.

Type 2 Diabetes – A chronic metabolic disorder due to insulin resistance and impaired glucose uptake, leading to complications like heart disease, nephropathy, and neuropathy.

Gestational Diabetes (GDM) – Occurs during pregnancy, increasing the risk of Type 2 Diabetes later[6-8].

Pathophysiology and Pathogenesis

Type 1 Diabetes involves beta-cell destruction by auto reactive T lymphocytes, leading to insulin deficiency.

Type 2 Diabetes is driven by insulin resistance, impaired insulin secretion, and increased fat breakdown.

Contributing factors include glucotoxicity, lipotoxicity, oxidative stress, and insulin resistance, leading to beta-cell dysfunction and diabetes progression.

Free fatty acids and high glucose levels impair insulin synthesis, trigger oxidative stress, and promote beta-cell exhaustion[9-10].

MATERIALS AND METHODS

Plant Collection and Extract Preparation

Fresh aerial parts of *Glinus lotoides* and *Mollugo cerviana* were collected from Usilampetty, Tamil Nadu, and authenticated at the Jawaharlal Nehru Tropical Botanical Garden. The dried plant material was powdered and subjected to cold maceration using hydro-alcoholic (70% ethanol) and absolute ethanol solvents. Extracts were concentrated using a vacuum evaporator, and yield percentage was calculated.

Phytochemical Screening

Qualitative tests were performed to detect carbohydrates, amino acids, glycosides, flavonoids, phenolic compounds, alkaloids, and steroids using standard protocols.

Anti-inflammatory Activity

In vitro protein denaturation inhibition assay was conducted using bovine albumin, and absorbance was measured at 660 nm. The percentage inhibition was calculated.

In vivo anti-inflammatory activity was evaluated using carrageenan-induced paw edema in Wistar rats. Groups received either plant extract (250 mg/kg, 500 mg/kg) or diclofenac (10 mg/kg) before induction. Paw thickness was measured at different intervals, and inflammation inhibition was calculated.

Evaluation of Plant Extracts for Anti-Diabetic Activity

In vitro α-Amylase Inhibition Assay

Glucose release from starch by α-amylase was measured using 3, 5-dinitrosalicylic reagent. The enzyme-inhibiting compounds reduced glucose production, assessed via a colorimetric assay at 405 nm.

In vivo Study on Nicotinamide-Streptozotocin (STZ) Induced Diabetic Rats

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- **Animals:** Male albino rats (150–200g) were housed under controlled conditions per CPCSEA guidelines.
- **Induction of Diabetes:** Rats were fasted and injected with 120 mg/kg nicotinamide (i.p.), followed by 60 mg/kg STZ after 15 minutes. Rats with glucose levels >250 mg/dL were selected.
- **Grouping & Treatment (28 Days):**
  - **Group I:** Normal saline
  - **Group II:** STZ + NIC
  - **Group III:** STZ + NIC + Glibenclamide (20 mg/kg)
  - **Group IV-V:** STZ + NIC + Extract I (Glinus lotoides, Low & High dose)
  - **Group VI-VII:** STZ + NIC + Extract II (Mollugo cerviana, Low & High dose)
- **Parameters Measured**
  - **Blood Glucose Levels:** Monitored using a glucometer.
  - **Body Weight:** Recorded periodically.
  - **Blood Sample Processing:** Retro-orbital blood collection for haematological and biochemical analysis.
  - **Tissue Homogenate Preparation:** Liver & kidney tissues homogenized for further assays.
  - **Haematological Parameters:** RBC, WBC, Differential leucocyte count, and Hemoglobin estimation.
  - **Serum Lipid Profile:** Cholesterol, triglycerides, HDL measured spectrophotometrically.
  - **Antioxidant Enzyme Assays:**
  - **Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Reduced Glutathione (GSH)** measured using colorimetric methods.
  - **Lipid Peroxidation (LPO):** TBARS assay at 532 nm.
  - **Hepatic Glucose Metabolism Enzymes:** Glycogen phosphorylase assay using phosphate buffer, glucose-1-phosphate, and glycogen as substrates.

Table 1: percentage yield of various extracts

Mollugo Cerviana Ser. (L)	
Percentage yield of Pet ether extract	2.8%w/w
Percentage yield of Ethanolic Extract	8.9%w/w
Glinus Lotoides (L)	
Percentage yield of Pet ether Extract	3.4%w/w
Percentage yield of Hydro alcoholic extract	12.1%w/w

RESULTS AND DISCUSSION

Percentage yield of various Plant Extracts

The percentage yield of various extracts of both the plants are

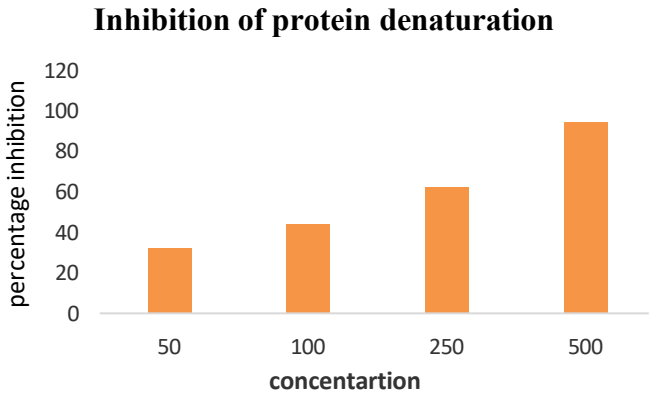


Figure 1: Inhibition of protein denaturation

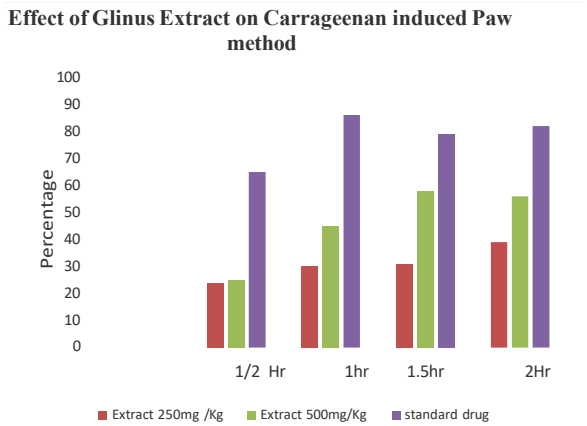


Figure 2: Carrageenan induced acute inflammation

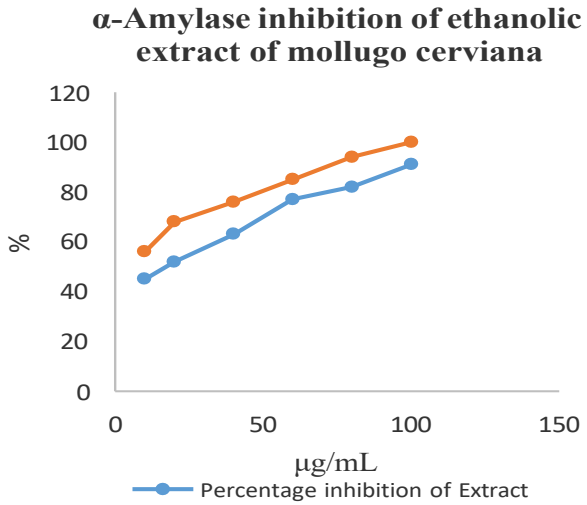


Figure 3: plot of concentration Vs Percentage inhibition

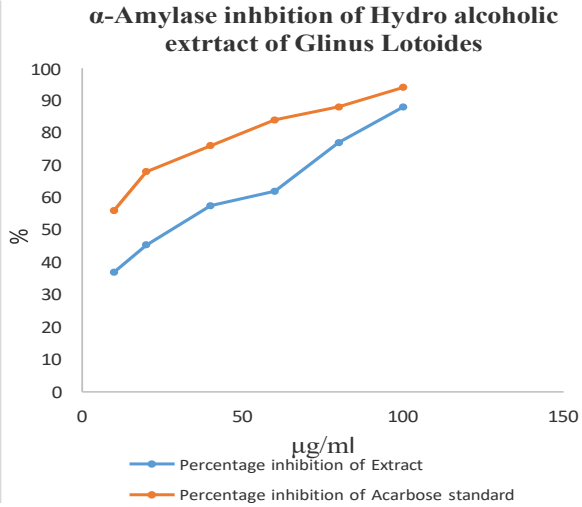


Figure 4: Plot of concentration Vs Percentage inhibition

Table 2: Phytochemical Evaluation of Plant Extracts

Phytochemical Constituent	Mollugo cerviana Ser. (L.) (Ethanolic extract)		Glinus Lotoides (L.) (Hydro--Alcoholic Extract)	
	Pet ether Extract	Pet ether Extract	Hydro-- alcoholic extract	Ethanolic extract
Carbohydrates	++	++	++	++
Proteins	++	++	++	++
Alkaloids	--	--	--	++
Tannins	--	--	--	++
Phenols	++	--	--	++
Flavonoids	--	--	++	++
Saponins	--	--	++	++
Glycosides	--	++	++	++
Terpenoids	--	--	--	++
Steroids	++	++	--	++

(++) Present; (--) Absent

tabulated. All the extracts were analyzed for secondary metabolites.

Preliminary Phytochemical screening

The results of preliminary phytochemical screening are depicted in Table No 2

Anti- Inflammatory activity of *Glinus Lotoides*

Invitro Anti- Inflammatory activity

Inhibition of protein denaturation

The hydro-alcoholic extract of Glinus lotoides was analysed for Inhibition of protein denaturation using salicylic acid as standard.

Table 3: Inhibition of protein denaturation

Concentration of Plant extract (µg/mL)	Percentage inhibition of protein denaturation (%)
50	32±2.4
100	44±0.76
250	62±0.67
500	94±1.3

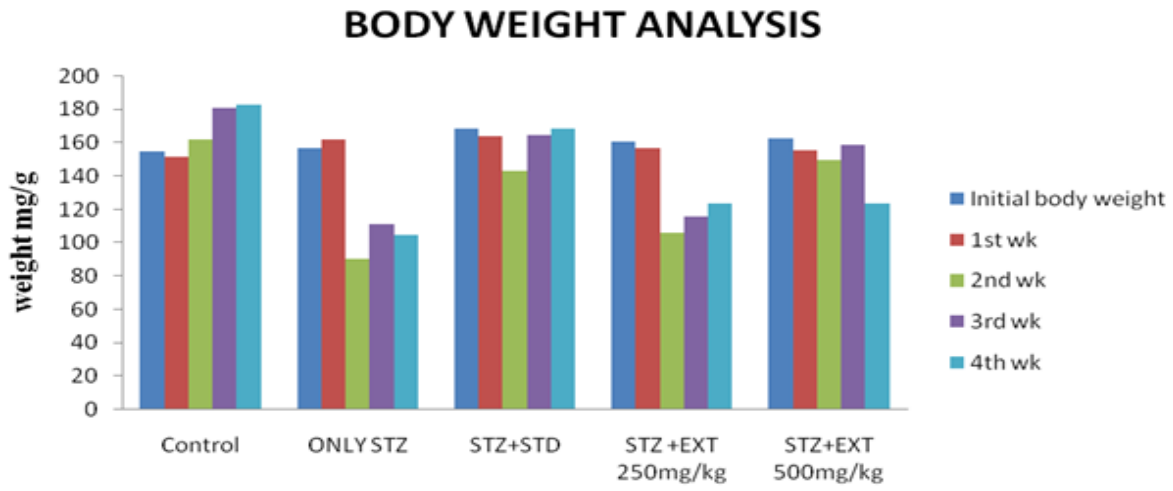


Figure 5: Body Weight analysis of Mollugo Cerviana

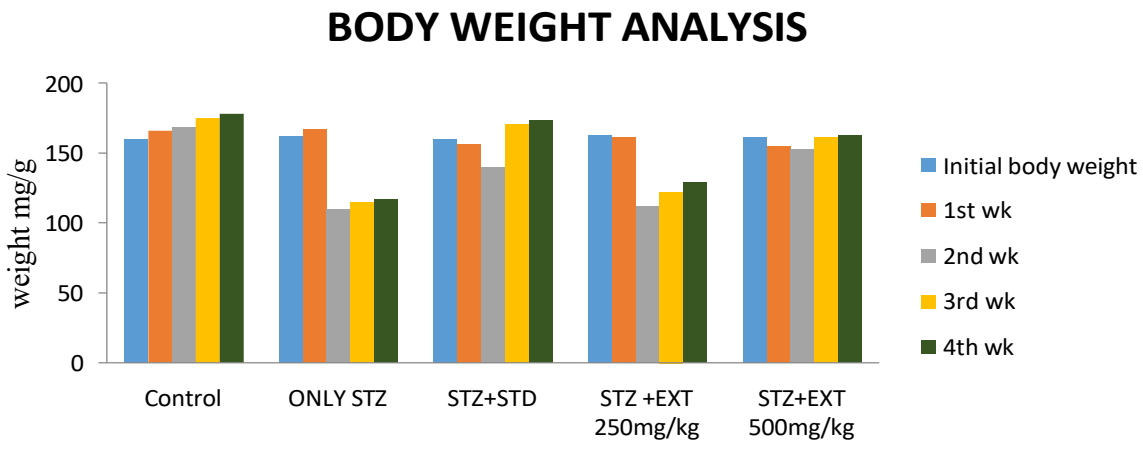


Figure 6: Body Weight analysis of Glinus Lotoides

Table 4: Anti-inflammatory activity of Glinus lotoides-Carrageenan induced paw method

Groups	% Inhibition 1/2 hr	%Inhibition 1 hr	% Inhibition 1 1/2 hr	%Inhibition 2 hr
CRGN+Hydo Alc extract 250	24.93±0.12	30.44±3.45	31.36±2.95	39.0±2.6
CRGN+Hydro Alc. Extract 500	25.6±0.99	45.1±4.17	50.9±3.67	56.34±1.5
CRGN + Diclofenac sodium	65.97±0.05	86.24±0.67	78.82±0.88	82.13±1.33

Values are expressed as means of standard deviation of 6 Rats

Table 5: α-Amylase inhibitory of Molllugo cerviana

Concentration	Percentage inhibition of Extract	Percentage inhibition of Acarbose standard
10	45±1.19	56±1.08
20	52±1.45	68±1.67
40	63±1.23	76±1.08
60	70±1.12	84±1.34
80	76±1.09	88±1.06
100	84±1.34	94±1.79

% inhibition value is mean ±SD

Table 6: α-Amylase inhibitory of GLL

Concentration µg/mL	Percentage inhibition of Extract	Percentage inhibition of Acarbose standard
10	37±0.99	56±1.08
20	45.5±0.04	68±1.67
40	57.5±0.43	76±1.08
60	62±0.94	84±1.34
80	77±0.45	88±1.06
100	88±1.08	94±1.79

The anti-inflammatory effect was evaluated by testing the extract's ability to prevent protein denaturation. Glinus lotoides showed significant inhibition of heat-induced albumin denaturation with an IC50 of 101.3 µg/mL. Aspirin was used as the standard drug.

In-vivo anti-inflammatory activity

Carrageenan-induced paw oedema model

Carrageenan-induced paw edema is a key method for evaluating anti-inflammatory agents, showing a biphasic response. The early phase (within an hour) is driven by injury, histamine, and serotonin release, while the second phase (third hour) involves cyclooxygenase (COX) and prostaglandin synthesis. Hydro alcoholic extract at 250 and 500 mg/kg reduced paw edema by 24.99% and 25.6% in the early phase, indicating no effect on histamine or serotonin. However, at the third hour, inhibition rates were 39.3% and 56.1%, suggesting COX inhibition, which may also help reduce pancreatic β-cell inflammation.

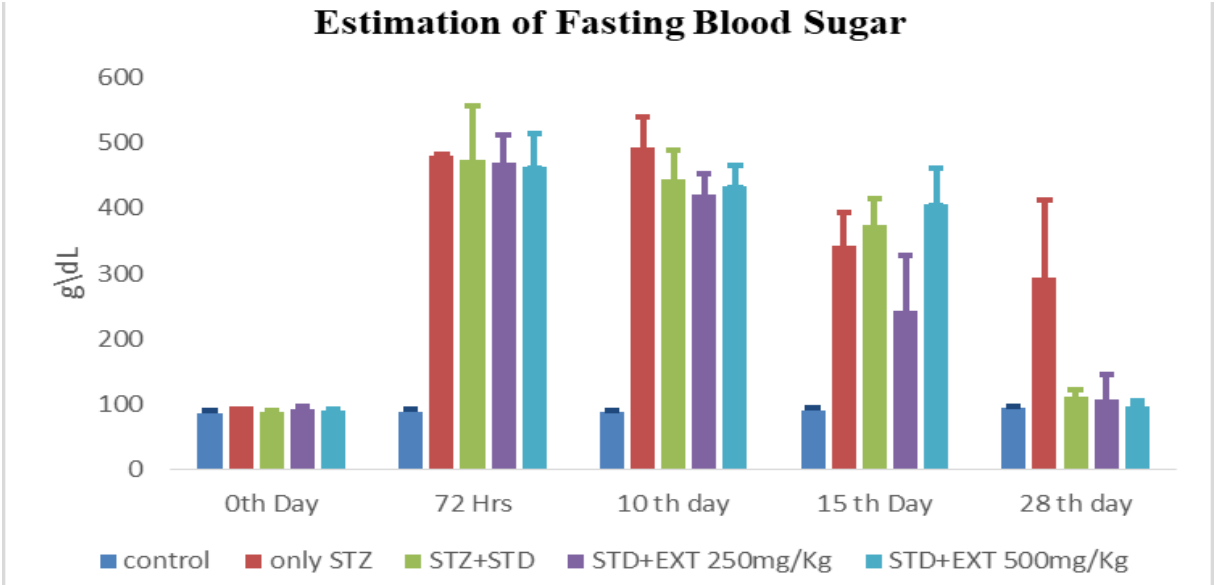


Figure 7: Analysis of mollugo cerviana on fasting blood sugar level

Table 7: Body weight analysis of STZ induced rats after treating with plant extract

Group	Before Induction of STZ	After Induction of STZ	Body Weight on diabetes animals with standard and extract		
	Initial Body weight	Body weight on 1st week	Body weight on 2nd week	Body weight on 3rd week	Body weight on 4th week
Control	160±0.65*	166±.93	168±0.799	175.8±0.85	178±1.648
ONLY STZ	162±1.12	162±1.63	90.8±32.1	111.5±39.47	105±37.4
STZ+STD	160±.93	164±1.41	143±1.7	164.8±1.702	169±1.25
STZ + MCR EXT 250mg/kg	163±0.32	161±0.98	112±5.28	122±14.17	125.9±41.4
STZ + MCR EXT 500mg/kg	161±2.02*	155±1.48	153±15.3	161±12.049	163±11.9
STZ + GLL EXT 250mg/kg	160±1.42	158±1.08	130±35.9	142±39.17	144±41.4
STZ + GLL EXT 500mg/kg	163±2.33*	156±2.74	150±2.9	2 .159±5.339	124±41.3

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing treated group with CONTROL group

Table 8: Effect of extracts on estimation of blood glucose in normal and experimental rats

Group	Before Induction of STZ		After Induction of STZ	Fasting blood sugar level on diabetes animals with standard and extract	
	Initial fasting blood sugar	Fasting blood sugar on 72 hr	Fasting blood sugar on 10th day	Fasting blood sugar on 15th day	Fasting blood sugar on 28th day
Control	86.5±3.95	88.5±4.05	88±3.63	90.5±4.11	94.5±2.63
ONLY ST	97±3.14ns	480±45.5***	493±49.9***	343±119ns	293±101
STZ+ST D	89.3±1.89ns	473±82.6***	345±44.3***	135±39.3*	113±8.54
STZ +MCR EXT 250mg/kg	93.5±3.62ns	470±41.4***	320±33.4***	243±84.6ns	118±36.8
STZ +MCR EXT 500mg/kg	90.8±2.39ns	463±51.1***	333±33.3***	175±56.8*	97.5±37.1
STZ +GLL EXT 250mg/kg	92.5±2.09ns	474±11.04***	332±2.4***	163±11.66ns	116±43.4
STZ +GLL EXT 500mg/kg	89.8±3.12ns	465±17.21***	253±24.5***	140±16.6*	109±31.9

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing treated group with CONTROL group

Table 9: Effect of extract on estimation of liver function test in normal and experimental rats

Group	Control	ONLY STZ	STZ+STD	STZ +EXT 250mg/kg	STZ+EXTII 500mg/kg	STZ +EXTI 250mg/kg	STZ+EXT 500mg/kg
SGPT (U/L)	51.8±1.44	124±0.629***	95±8.44***	111±1.47***	87.5±3.59***	108±3.12	95±4.04
SGOT (U/L)	44±2.27	42.5±0.645ns	34.8±1.11**	44.8±3.43ns	29±1.29***	55±1.99	32±1.04
ALP (U/L)	131±3.3	142±5.79	137±17.3	138±2.95	128±3.12	135±1.39	130±2.69

In-Vitro Anti diabetic activity Studies

α-Amylase inhibition Assay

α-Amylase inhibitory of Ethanolic extract of *Mollugo cerviana* (*Ser) L*

The ethanolic extract of *Mollugo cerviana* exhibited a concentration-dependent inhibition of α-amylase, with an

IC50 of 13.5 µg/mL, compared to 10 µg/mL for the acarbose standard. Analysis using GraphPad software confirmed the extract's significant inhibitory activity, suggesting its potential as a natural alternative for enzyme inhibition.

α-Amylase inhibitory of Hydro-alcoholic extract of *Glinus Lotoides* (L)

Table 5.6 shows that the hydroalcoholic extract exhibited dose-dependent α-amylase inhibition. Figure 5.4 presents the results compared to the acarbose standard. IC50 values were 12.5 µg/mL for *Glinus lotoides* extract and 10 µg/mL for acarbose, as calculated using GraphPad software.

Evaluation of Anti-diabetic Activity of Plant extracts by *In vivo* Methods

STZ and nicotinamide together induce mild, stable hyperglycemia by reducing pancreatic insulin reserves.

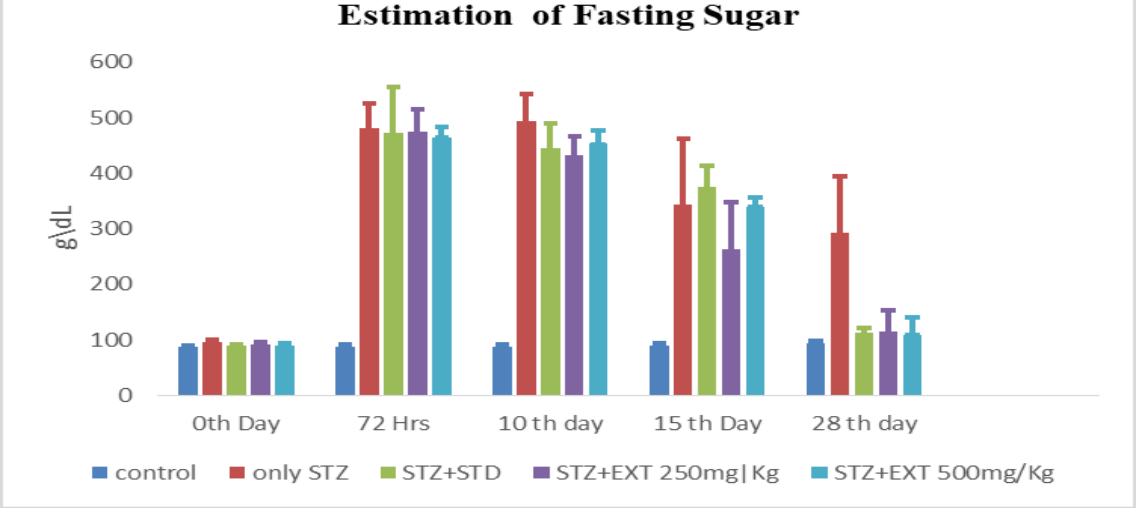


Figure 8: Analysis of Glinus extract on fasting blood sugar level

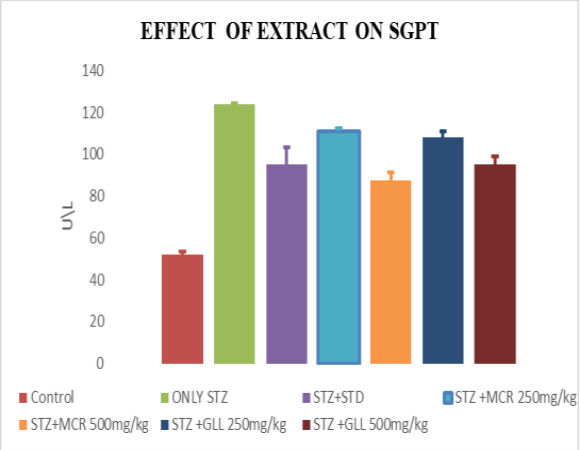


Figure 9: Effect of Plant extract on SGPT

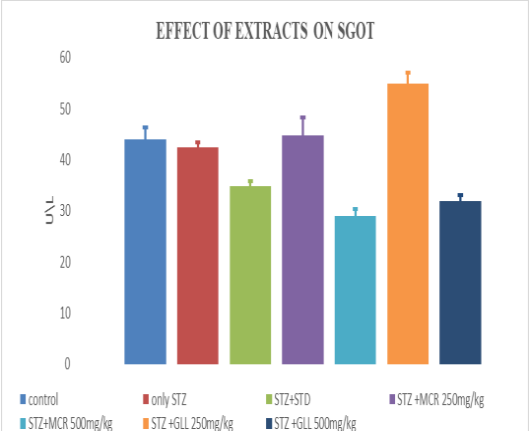


Figure 10: Effect of Plant extract on SGOT

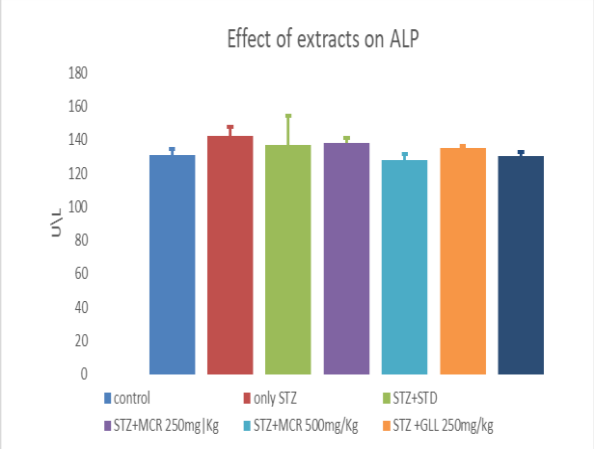


Figure 11: Effect of Plant extract on SGPT

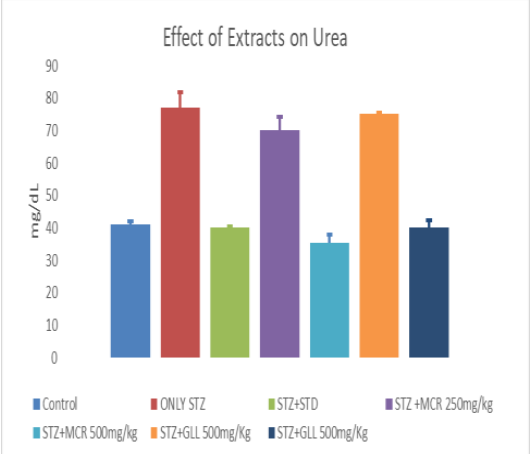


Figure 12: Effect of Plant extracts on Urea

Table 10: Effect of extract on estimation of kidney function test in normal and experimental rats

Group	Control	ONLY STZ	STZ+ST D	STZ MC 250 g	R + STZ + MCR 500 mg / Kg	STZ + GLL 250 g/ Kg	STZ + GLL 500 mg/ Kg
Urea (mg/dl	41±1.08	77±4.8***	40±0.408 ns	70±4.16***	35.3±2.56	75.3±2.23	40.3±2.07^{ns}
Uric acid (mg/dl)	0.5±0.06	1.73±0.085	0.775±0.06	1.5±0.129	0.57±0.048	1.64±0.129	0.683±0.008
Creatinine (mg/dl)	0.62±0.10	1.8±0.02**	0.8±0.040^{ns}	1.5±0.17***	0.57±0.075	1.56±0.005**	0.568±0.008^{ns}
PROTEIN (mg/dl)	5.31±0.59	9.2±0.0897***	7.26±0.104**	8.5±0.505***	6.86±0.18*	7.6±0.674***	6.86±0.108*

Table 11: Effect of extract on estimation of lipid profiles in normal and experimental rat

Group	Control	ONLY STZ	STZ+STD	STZ + MCR 250mg/kg	STZ+MCR 500mg/kg	STZ + GLL 250mg/kg	STZ+GLL 500mg/kg
Total Cholestrol (mg/dl)	48.9±2.33	54.4±1.55^{ns}	48±0.592**	52.7±2.18^{ns}	49.9±1.47*	51.81±1.81^{ns}	48.23±2.07*
Triglycerides (mg/dl)	53.2±4.79	100±0.92***	71.7±1.1^{ns}	86.4±6.38**	55.8±7.57^{ns}	94.4±6.38**	70.8±7.57^{ns}
VLDL-Cholestrol (mg/dl)	8.53±0.47	11.5±0.25**	7.4±0.694^{ns}	10.6±0.358^{ns}	8.8±0.749^{ns}	10.4±0.358^{ns}	6.8±0.749^{ns}
LDL - Cholestrol (mg/dl)	16.3±1.66	21±2.14^{ns}	12.9±1.03^{ns}	18.4±0.941^{ns}	15.3±1.87^{ns}	18.4±0.941^{ns}	14.93±1.87^{ns}
HDLCholesterol (mg/dL)	24.07±0.9	21.9±1.09**	27.7±2.22*	23.7±1.1*	25.8±2.01*	23.01±1.45*	25.4±0.98*

Values are communicated as the mean ± S.D; Factual importance (p) determined by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 determined by contrasting treated gathering and CONTROL bunch

Table 12: Effect of Extracts on Liver Glycogen

Group	Cont rol	ONLY STZ	STZ+STD	STZ+M CR 250mg/k g	STZ+M CR 500mg/k g	STZ+GL L 250mg/kg	STZ+GL L 500mg/kg
Glycog en (mg/dl)	33.3 ±1.59	20.8±0.302**	32.7±2.95ns	24.7±3.95*	30.5±1.08ns	21.4±1.45***	32.2±0.845**

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing treated group with CONTROL group

STZ enters pancreatic cells via GLUT2, damaging DNA and triggering excessive polymerase activity, leading to NAD(+) and ATP depletion, and ultimately, cell death. STZ also causes kidney damage, oxidative stress, and inflammation. Nicotinamide inhibits PARP activity, limiting tissue necrosis and maintaining controlled hyperglycemia, effectively mimicking diabetes while preserving insulin sensitivity.

Effect of extract on body weight analysis in normal and experimental rats

STZ-nicotinamide-injected rats showed significant weight loss due to muscle wasting and impaired glucose metabolism, leading to polyuria, polydipsia, and polyphagia. Chronic glucose excretion and reduced glycogen synthesis further prevented weight gain. Body weight measurements over four weeks revealed a significant ($p<0.001$) decrease in diabetic rats compared to the normal control. However, treatment with Mollugo cerviana ethanolic extract, Glinus hydroalcoholic extract (250 mg/kg, 500 mg/kg), and glibenclamide (20 mg/kg) significantly improved body weight from the second to fourth week ($p<0.001$, $p<0.05$, $p<0.01$).

No mortality or adverse reactions were observed in diabetic and treated animals. While diabetic control rats experienced significant weight loss, those treated with plant extracts and glibenclamide showed notable weight gain, likely due to reduced muscle atrophy and improved insulin activity. After 28 days, metabolic abnormalities were resolved, possibly due to enhanced insulin release and insulin-mimicking effects. The extracts also helped prevent protein breakdown by reversing gluconeogenesis, as indicated by moderate weight gain improvement. Lower serum total protein in diabetic rats suggested an increased amino acid conversion to glucose, further supporting the plant extracts' regulatory effects.

Effect of extracts on estimation of blood glucose in

Table 13: Effect of extract on glycosylated hemoglobin

Group	Control	ONLY STZ	STZ+S TD	STZ +MCR 250mg/ kg	STZ+MC R 500mg/kg	STZ+ GLL 250m g/kg	STZ+G LL 500mg/ kg
HbA1c (mmol/l)	4.78±0. 249	11.6±0.4 22***	5.38±0.1 16^{ns}	9.19±0.1 6***	6.48±0.31 9^{ns}	8.78± 0.32***	6.98±0.4 3**

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing treated group with CONTROL group

Table 14: Effect of extract on estimation of enzymatic, non- enzymatic antioxidant and lipid-peroxidation levels in normal and experimental rats

Group	Control	ONLY STZ	STZ+STD	STZ +MCR 250mg/kg	STZ+MCR 500mg/kg	STZ+GLL 250mg/kg	STZ+GLL 500mg/kg
SOD	3.49±0. 10	2.65±0.053 9^{ns}	6.12±0.576* **	2.2±0.0674*	6.16±0.35***	2.9±0.091*	6.16±0.25***
CATALA SE	65.1±6. 1	27.6±0.333 ***	90.7±0.44** *	30.5±0.91***	83.4±3.2**	40.5±0.57***	76.4±0.56**
GSH	8.37±0. 14	7.3±0.0682 ***	9.42±0.0125 **	7.56±0.222**	8.92±0.23^{ns}	7.9±0.104 **	9.42±0.78^{ns}
LPO	24.7±2. 0	31.5±0.203 **	16±0.793***	27.3±0.372^{ns}	17.7±1.68**	29.3±0.803^{ns}	19.7±0.65**

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing treated group with CONTROL group

Table 15: Effect of extract on the levels of carbohydrate metabolizing enzymes in normal and experimental rats

Group	Control	ONLY STZ	STZ+ST D	STZ+EXT 250mg/kg	STZ+EXT 500mg/kg	STZ+EXT 250mg/kg	STZ+EXT 500mg/kg
Glucokinase	16.5±0. 3	8.13±0.17 ***	15.3±0.31 5^{ns}	9.51±0.486**	14.6±0.503*	14.3±0.315^{ns}	16.5±0.408**
Hexokinase	4.2±0.0 3	2.42±0.08 0**	3.99±0.06 0^{ns}	3.02±0.0524* *	3.89±0.13*	2.99±0.0606^{ns}	4.02±0.054**
Glucose-6-Phosphatase	7.1±0.8 0	44.5±8.81 ***	29.5±3.51 *	41.7±1.65***	25.8±1.74*	42.56±3.51*	24.54±1.05**
Fructose 1--6-di Phosphatase	69.1±0. 77	32.6±0.03 **	61.1±3.06 ^{ns}	45.9±3.889**	48.96±4.69**	52.6±1.99^{ns}	60.9±2.092*

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ***P< 0.001, **P < 0.01,*P < 0.05 calculated by comparing treated group with CONTROL group

normal and experimental rats

Plant extracts help lower blood sugar by restoring β-cell function, reducing inflammation, and enhancing insulin secretion. They also limit glucose absorption in the intestine and support insulin dependent processes. Findings are summarized in Table 9.

Hyperglycemia results from increased glycogenolysis, gluconeogenesis, and impaired glucose uptake due to insulin deficiency. STZ+NIC administration caused a fivefold rise in fasting glucose levels, maintained for three weeks.

After 28 days of treatment with glibenclamide and plant extracts (250 mg/kg, 500 mg/kg), glucose levels dropped by 23-72% dose-dependently. The 500 mg/kg ethanolic extract of Mollugo cerviana showed the most significant reduction, likely due to increased insulin production from pancreatic β-cells. Similar effects have been observed with other antihyperglycemic plants.

Effect of the extract on liver function test estimation in normal and experimental rats

The liver plays a crucial role in glucose homeostasis, and liver function tests (LFTs) help monitor liver health, especially in type 2 diabetes patients. Key LFTs include ALT and AST (hepatocyte damage markers), ALP, GGT, and bilirubin (biliary function and cholestasis indicators), and albumin and prothrombin (protein synthesis markers). Mild transaminase elevation often signals insulin resistance, with chronic hyperinsulinemia contributing to liver insulin resistance. Table 5.9 presents the findings on these key liver function markers.

Diabetic rats showed significantly elevated liver marker enzymes (ALT, AST, ALP) due to STZ-induced hepatocyte damage and oxidative stress. ALP, a key enzyme in liver function, was also elevated, indicating hepatic insulin resistance. Treatment with Glibenclamide and plant extracts (Glinus lotoides, Mollugo cerviana) restored enzyme levels, suggesting their potential in protecting liver cells and aiding regeneration.

Effect of extract on estimation of kidney function test in normal and experimental rats

Diabetic nephropathy, linked to oxidative stress, leads to renal dysfunction with increased urea, creatinine, uric acid,

Table 16: Various fractions of Mollugo cerviana

1.	Chloroform	100	1 ,2,3,4
2.	Chloroform: Ethyl acetate	80:20	5,6,7, 8
3.	Chloroform: Ethyl acetate	60:40	9,10,11,12
4.	Chloroform: Ethyl acetate	40:60	13,14,15,16
5.	Chloroform: Ethyl acetate	20:80	17,18,19,20
6.	Ethyl acetate	100	21,22,23,24
7.	Ethyl acetate :Ethanol	80:20	25, 26,27,28
8.	Ethyl acetate: Ethanol	60:40	29,30,31,32
9.	Ethyl acetate: Ethanol	40:60	33,34,35,36
10.	Ethyl acetate: Ethanol	20:80	37,38,39,40
11.	Ethanol	100	41,42,43,44

Table 17: TLC Profile of Fractions Collected in Column Chromatography

Minor fraction	Major fraction	No of spots	Rf value
1 -3	1	0	0
4-5	2	1	0.73
6-7	3	2	0.63 , 0.88
8-12	4	1	0.67
12-15	5	1	0.44
15-19	6	2	0.56, 0.71
20-28	7	0	0
29-31	8	1	0.8
32-34	9	0	0
35-40	10	1	0.45
41-44	11	0	0.88

Table 18: Inhibitory effect of Various Solvent Eluent on α-Amylase

Major Fraction	Percentage inhibition
1	1.28±0.98%
2	3.23±1.99%
3	3.12±0.45%
4	1.67±1.19%
5	2.21±0.98%
6	2.08±1.9%
7	23.53 ± 0.93%
8	62.69 ± 0.19%
9	11.23±1.02%
10	3.12±0.78%
11	1.18±0.09%

and proteinuria in STZ-induced diabetes. Hyperglycemia-induced kidney damage can progress to ESRD. Mollugo cerviana and Glinus lotoides extracts showed efficacy in preventing early kidney impairment, as seen in Table 5.11

In the trial's early days, glycosuria, polyuria, and elevated urea, uric acid, and creatinine were observed. Glibenclamide restored normal renal parameters, and Mollugo cerviana and Glinus lotoides extracts also normalized these levels, showing renal-protective properties.

Effect of extract on estimation of lipid profiles in normal and experimental rats

Hyperlipidemia, which is characterised by greater TC, TG, and LDL levels as well as a drop in high-density lipoprotein levels, is usually related with hyperglycemia. A failure in lipid metabolism causes a high prevalence of cardiovascular morbidity and mortality in type 2 diabetes.[138-141]

Table 5.10 shows increased triacylglycerol, total cholesterol, VLDL, and LDL, with decreased HDL in diabetic rats. Mollugo cerviana and Glinus lotoides extracts reduced VLDL, LDL, and triglycerides, with Mollugo cerviana (500 mg/kg) effectively normalizing VLDL levels ($p<0.001$).

Table 19: Various fractions of Glinus lotoides

1.	Hexane	100	1,2,3,4
2.	Hexane : Chloroform	90 : 10	5,6,7,8
3.	Hexane : Chloroform	80:20	9,10,11,12
4.	Hexane : Chloroform	70:30	13,14,15,16
5.	Hexane : Chloroform	60:40	17,18,19,20
6.	Hexane : Chloroform	50:50	21,22,23,24
7.	Hexane : Chloroform	40:60	25,26,27,28
8.	Hexane : Chloroform	30:70	29,30,.31,32
9.	Hexane : Chloroform	20:80	33,34,35,36
10.	Hexane : Chloroform	10:90	37,38,39,40
11.	Chloroform	100	41,42,43.44
12.	Chloroform: Ethyl acetate	90: 10	45,46,47,48
13.	Chloroform: Ethyl acetate	80:20	49,50,51,52
14.	Chloroform: Ethyl acetate	70:30	53,54,55,56
15.	Chloroform: Ethyl acetate	60:40	57,58,59,60
16.	Chloroform: Ethyl acetate	50:50	61,62,63,64
17.	Chloroform: Ethyl acetate	40:60	65,66,67,68
18.	Chloroform: Ethyl acetate	30:70	69,70,71,72
19.	Chloroform: Ethyl acetate	20:80	73,74,75,76
20.	Chloroform: Ethyl acetate	10:90	77,78,79,80
21.	Ethyl acetate	100	81,82,83,84

Effect of Extracts on Liver Glycogen

The liver regulates glycogen synthesis and buffers postprandial hyperglycemia.

In STZ- induced diabetic rats, glycogen synthase activation was inhibited, reducing glycogenesis.

The hepatic glycogen content of diabetes fed rats is rather the liver glycogen level was

considerably lowered (P0.05) in diabetic rats, whereas those given with both leaf extracts kept the glycogen level at the same level as control rats.

Glycosylated haemoglobin and the effect of the extract

Insulin has an anabolic effect on protein metabolism, meaning it speeds up protein synthesis while decelerating protein breakdown that can explain why diabetic rats have decreased haemoglobin levels. In uncontrolled or poorly controlled diabetes, a number of proteins, include haemoglobin, are glycosylated more often. HbA1c is a reliable indication of glycemic control in diabetics.

20 Effect of extract on glycosylated hemoglobin

Treatment with Mollugo cerviana and Glinus Lotoides

extracts normalized HbA1c, indicating effective glycemic control and potential anti-diabetic properties.

Antioxidant enzymes prevent harmful oxidative intermediates. In diabetes, decreased SOD and CAT activity leads to lipid peroxidation. STZ-induced diabetic rats showed inhibited enzyme activity due to glycation and free radicals.

In diabetes, excess ROS buildup leads to oxidative stress and lipid peroxidation, reducing SOD and GSH-Px activity. STZ-induced diabetic rats treated with Mollugo cerviana and Glinus Lotoides extracts (500 mg/kg) for 21 days showed improved antioxidant levels. The presence of Apigenin and Vitexin flavonoids helped neutralize free radicals and restore antioxidant defenses.

Effect of extract on the levels of carbohydrate metabolizing enzymes in normal and experimental rats

The main causes of diabetes mellitus (DM) are oxidative stress and blood glucose homeostasis. Significant alterations in the principal carbohydrate metabolising enzymes characterise the pathological conditions of metabolic disease. The level of hepatic GK was considerably lowered in streptozotocin (STZ)-induced diabetic rats, although the activities of G6Pase and fructose-1, 6-bisphosphatase were significantly elevated. As a result, boosting glycolysis by specifically targeting metabolic and regulatory enzymes could be a viable diabetic treatment

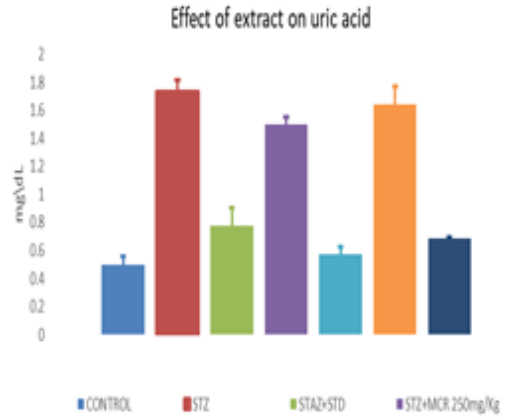


Figure 13: Effect of Plant extracts on Uric Acid

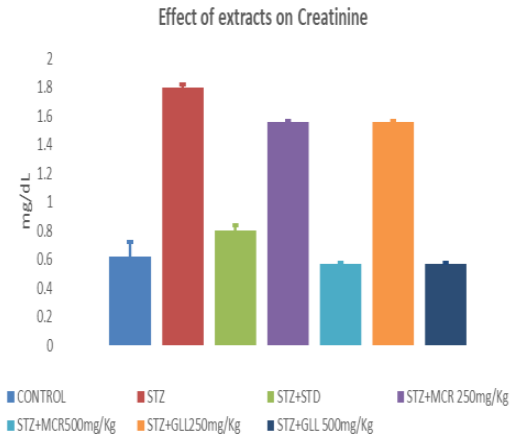


Figure 14: Effect of Plant extracts on Creatinine

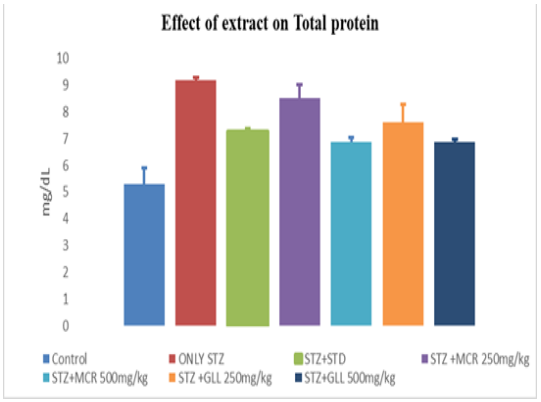


Figure 15: Effect of Plant extracts on Total Protein

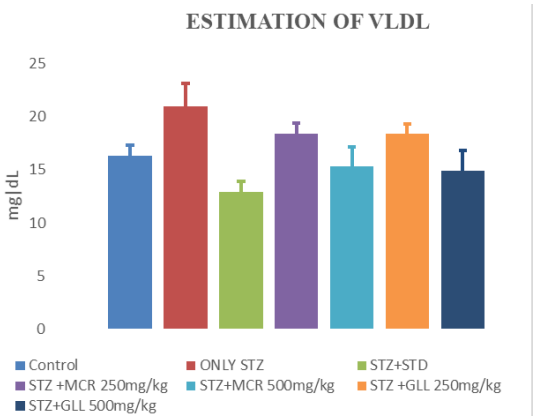


Figure 16: Effect of Plant extract on VLDL

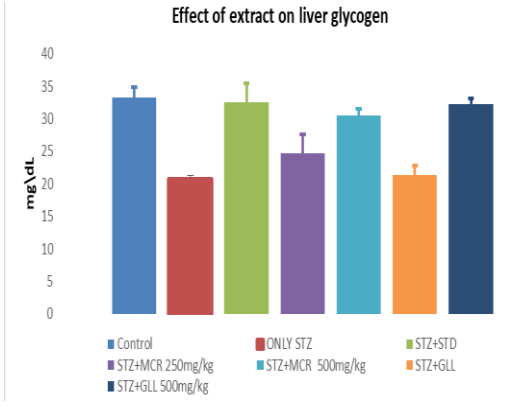


Figure 17: Effect of Extract on Liver Glycogen

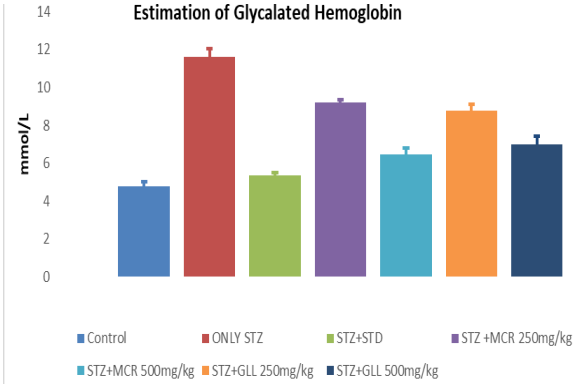


Figure 18: HbA1c levels were elevated in diabetic rats and correlated with fasting blood glucose.

Table 20: TLC Profile of Fractions Collected in Column Chromatography

Minor fraction	Major fraction	No of spots	Rf value
1 -6	1	0	0
7-8	2	1	0.68
9-14	3	0	0
15-18	4	1	0.77
19-25	5	1	0.64
26-31	6	2	0.66, 0.81
31-37	7	0	0
38-40	8	1	0.74
41-44	9	0	0
45-47	10	1	0.68
48-50	11	0	0
51-57	12	1	0.64
58-60	13	3	0.62,0.87,0.79
61-63	14	1	0.78
64-68	15	0	0
69-71	16	1	0.88
72-82	17	0	0.68,0.81,0.76
82-84	18	0	0

approach.

Streptozotocin reduced Glucokinase and Hexokinase activity, but plant extracts restored enzymatic function by activating mRNA coding. In diabetes, increased Fructose-1,6-bisphosphatase and G6Pase activity leads to excess glucose production. Plant extracts normalized these enzyme levels, indicating a balance in glycolysis and gluconeogenesis, similar to standard treatment.

Table 21: Inhibitory effect of Various Solvent Eluent on α-Amylase

Major Fraction	Percentage inhibition
2	1.11±0.23%
4	5.21±0.87%
5	1.31±0.75%
6	76.54±0.22%
8	3.21±0.11%
10	1.32±0.98%
12	1.35 ± 0.86%
13	0.69 ± 0.12%
14	5.23±0.82%
16	0.89±0.18%
17	1.23±0.23%

Table 22: Docking Score and Binding Energy of the Compounds with the Proteins

Protein-Ligand complex	Affinity Kcal/mol
α-Amylase-vitexin	-7.7
α-Amylase-acarbose	-8.7
α-Glucosidase-vitexin	-8.3
α-Glucosidase-Acarbose	-9.1
GLUT-4-vitexin	-9.5
GLUT-4-pioglitazone	-9.3
PPAR-γ-vitexin	-9.1
PPAR-γ-pioglitazone	-9.6

Effect of plant extracts on Histology

Histological changes in the pancreatic cell of normal control, diabetic control and treated rats were observed.

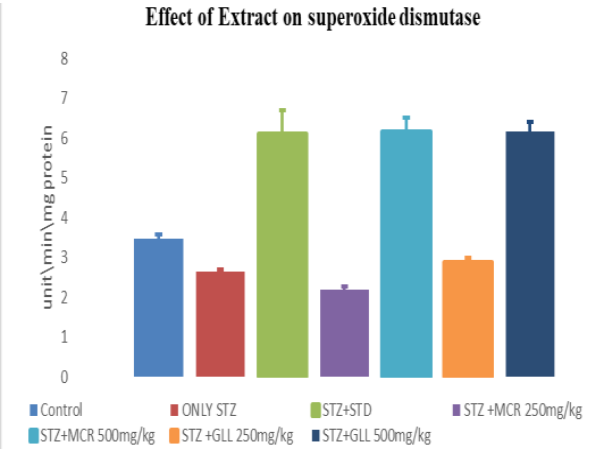


Figure 19: Effect of extracts on superoxide dismutase

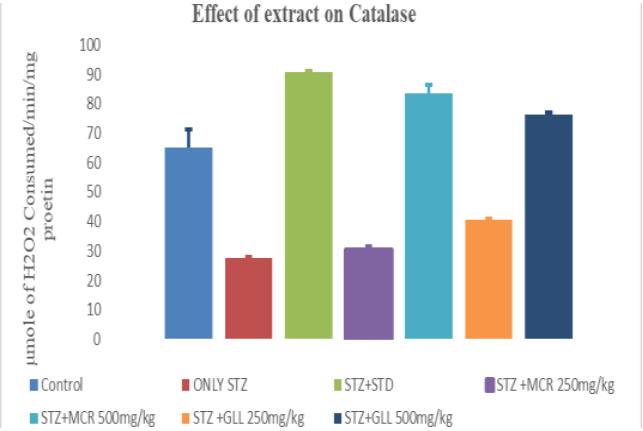


Figure 20: Effect of extracts on catalase

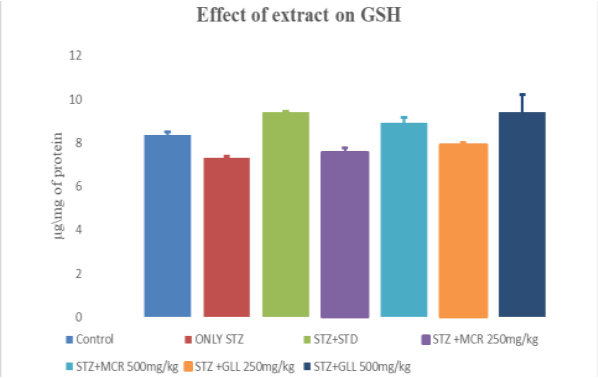


Figure 21: Effect of extracts on GSH

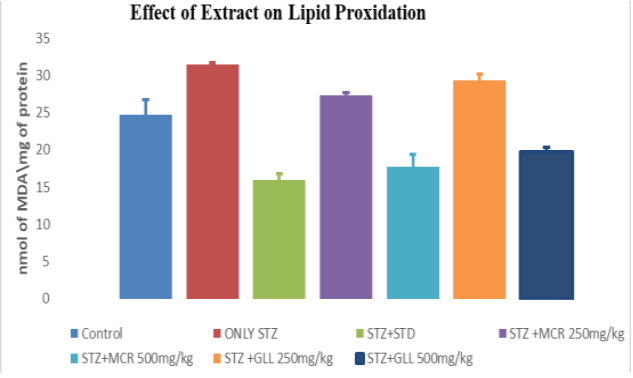


Figure 22: Effect of extracts on Lipid peroxidation

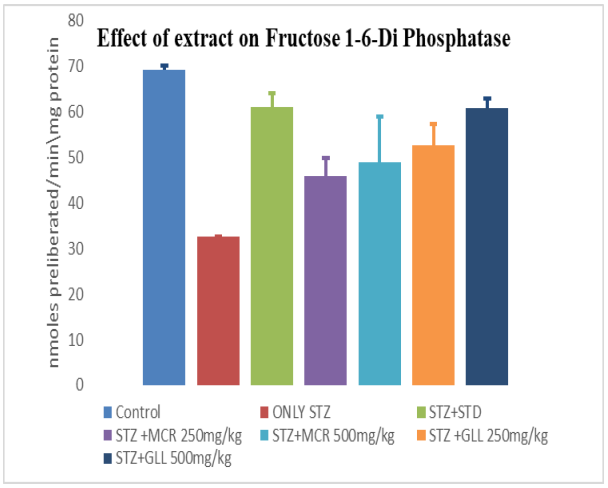


Figure 23: Effect on Fructose 1,6 Di Phosphatase

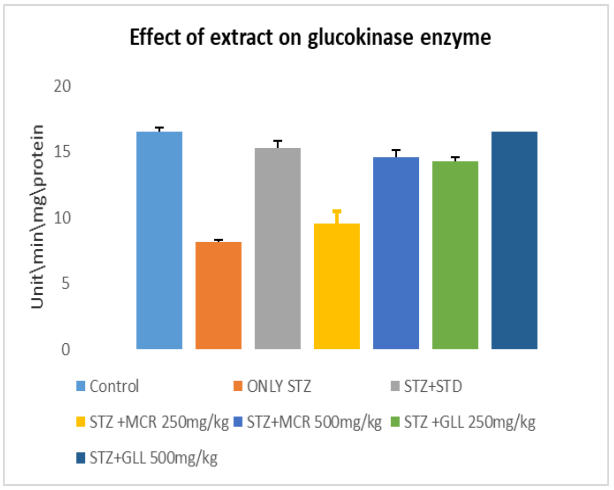


Figure 24: Effect of extracts on Glucokinase

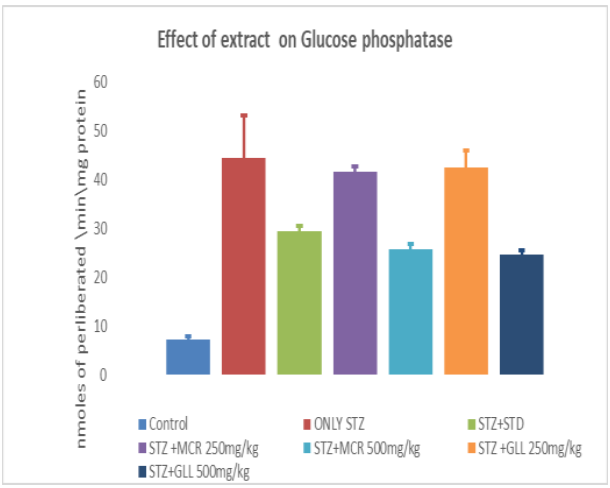


Figure 25: Effect of extracts on Glucose phosphatase

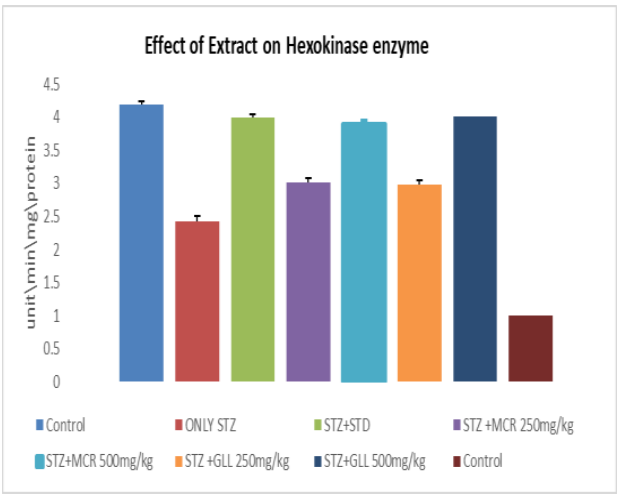


Figure 26: Effect of extracts on Hexokinase

MICROSCOPIC APPEARANCE

Normal pancreatic acini with minor lymphocytic infiltrates in a section from the pancreas. Islets have a typical size and a low number. There are no substantial pathologies in the blood vessels.

MICROSCOPIC APPEARANCE

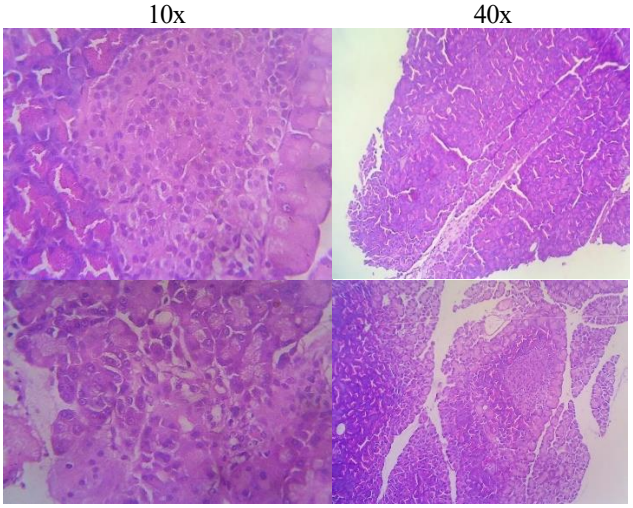
Pancreatic acini having inflammatory infiltrates in a section of the pancreas. Islets have a focal immolated architecture with lymphocytic infiltrates, and there are fewer and smaller islets with one focal destructed architecture. Vacuolation of the cytoplasm. There are no substantial pathologies in the blood vessels.

MICROSCOPIC APPEARANCE

Normal pancreatic acini with focal minor lymphocytic

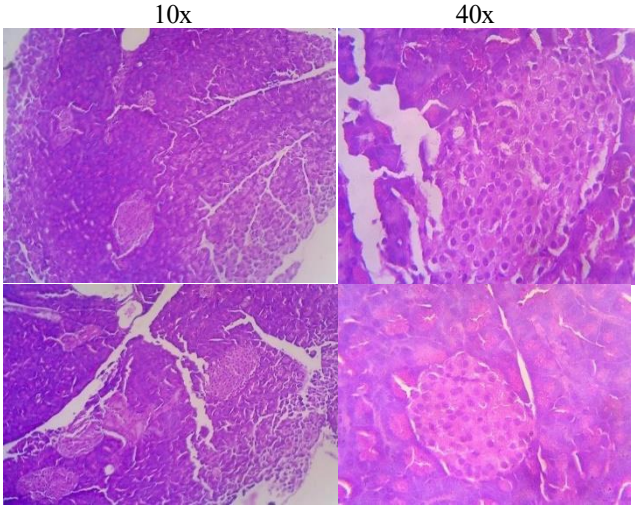
GROUP-I CONTROL

10x 40x



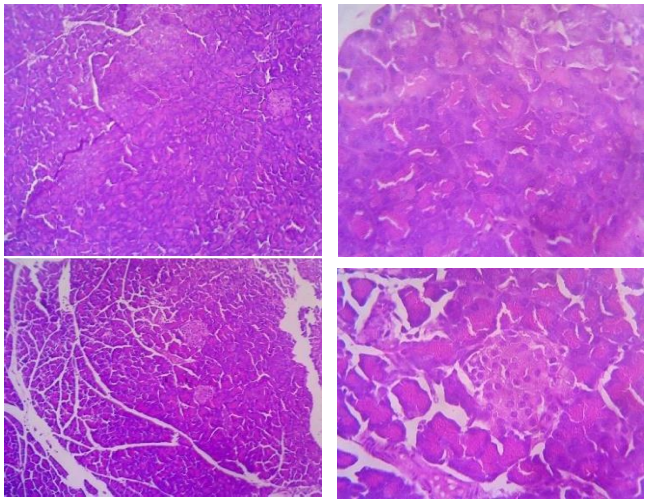
GROUP-II ONLY STZ

10x 40x



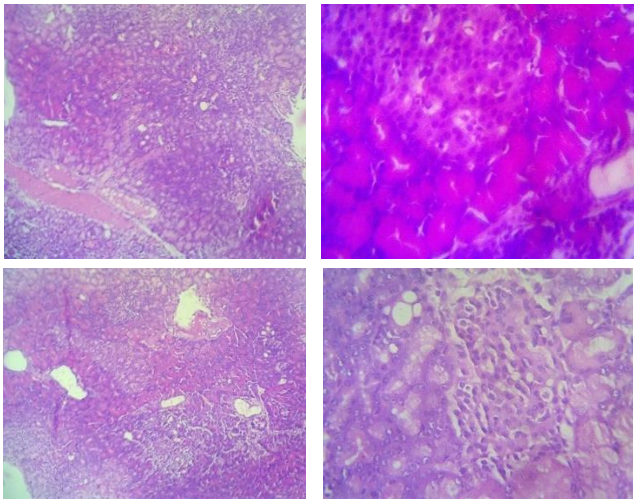
GROUP-III STZ + STD

10x 40x

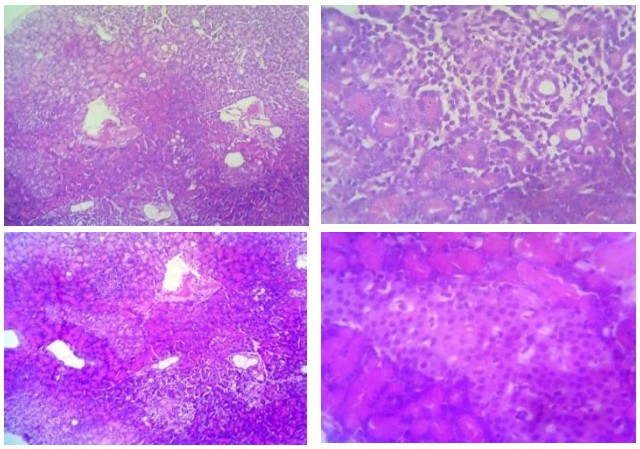


GP-IV- STZ + MCR EXT 250 mg/kg

10x 40x

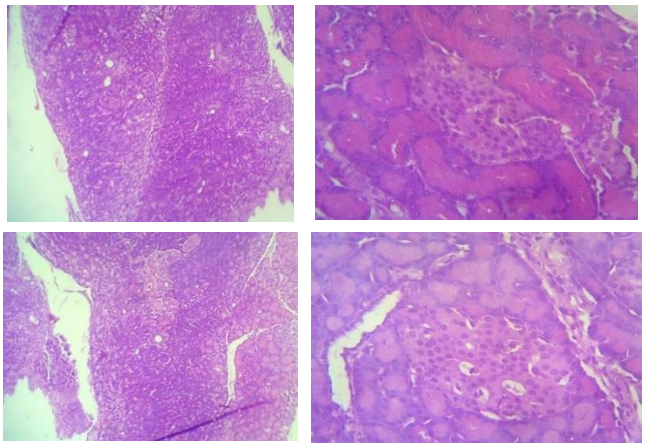


GP-IV- STZ +GLL EXT 250 mg/kg



GP-IV- Treated group
STZ + MCR EXT 500 mg/kg

10x 40x



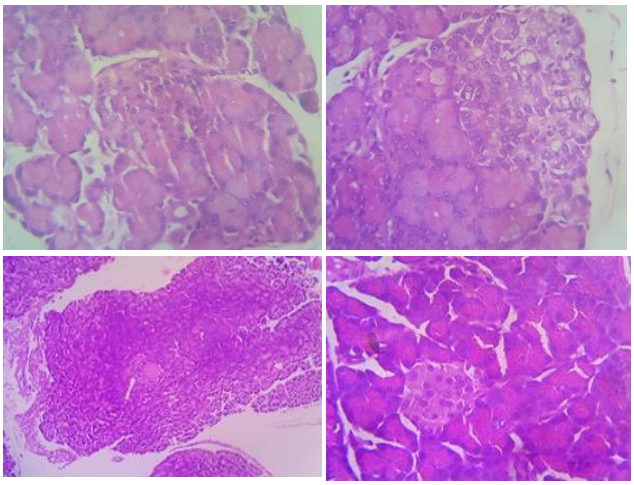
infiltrates in a pancreas section. A few islets with normal size and minor cytoplasmic vacuolation are also detected. The blood vessels appear to be normal.

MICROSCOPIC APPEARANCE

Pancreatic acini with lymphocytic infiltrates are shown in a section of the pancreas. The number and size of the islets are normal, with some lymphocyte infiltration and localised cytoplasmic vacuolation. There is no major disease in the blood vessels.

The pancreas supports the digestion of glucose and lipids. STZ treatment and oxidative pressure created by changed digestion make harm pancreatic cells. As indicated by our discoveries, necrotized pancreatic cells could be resuscitated under the effect of plant separates. In diabetic control rodents, myocyte clog is self-evident. The utilization of these plant removes diminishes clog through modifying glucose and lipid digestion. The harmfulness of STZ is shown by the obliteration of islet cells in the pancreas of diabetic control rodents. At the point when diabetic rodents were given plant separates, the harms and poisonousness were diminished somewhat, contrasted with diabetic control rodents which are not given plant removes.

GP-IV- STZ +GLL EXT 500 mg/kg



Bioactive Guided Separation, Isolation and Identification of Active Principle by Analytical Techniques

Separation of Active Phytoconstituents by Silica Gel Column Chromatography

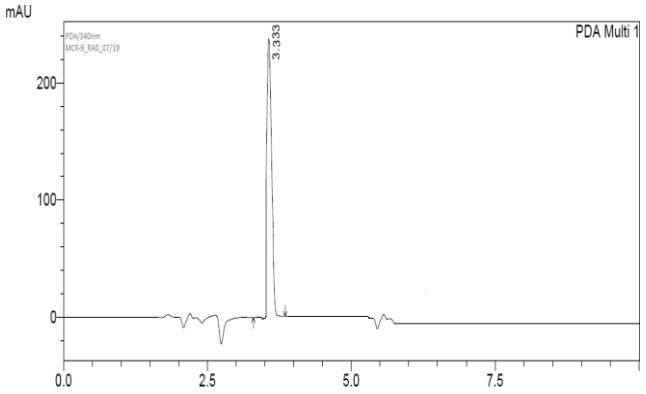


Figure 27: Chromatogram of the Isolated Pure Fraction I

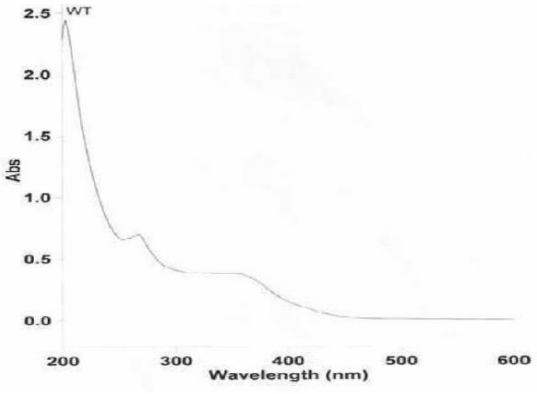


Figure 28: UV Spectrum of Isolated fraction

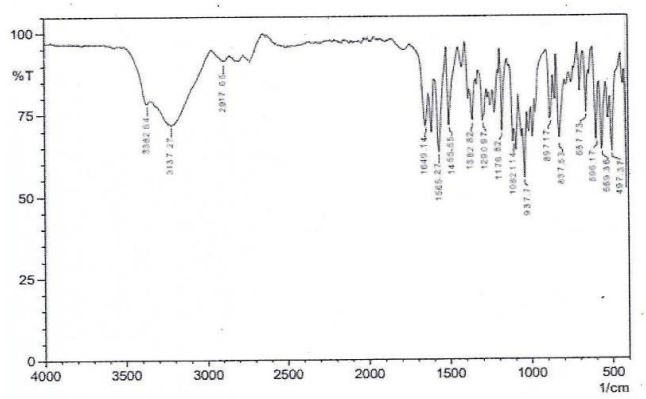


Figure 29: FTIR spectrum of isolated compound

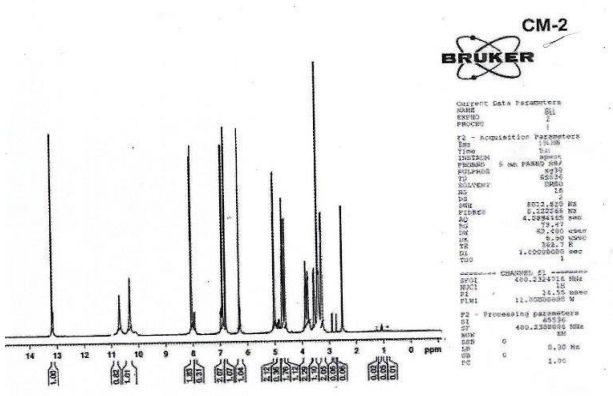


Figure 30: NMR Spectra of the Isolated Pure Compound

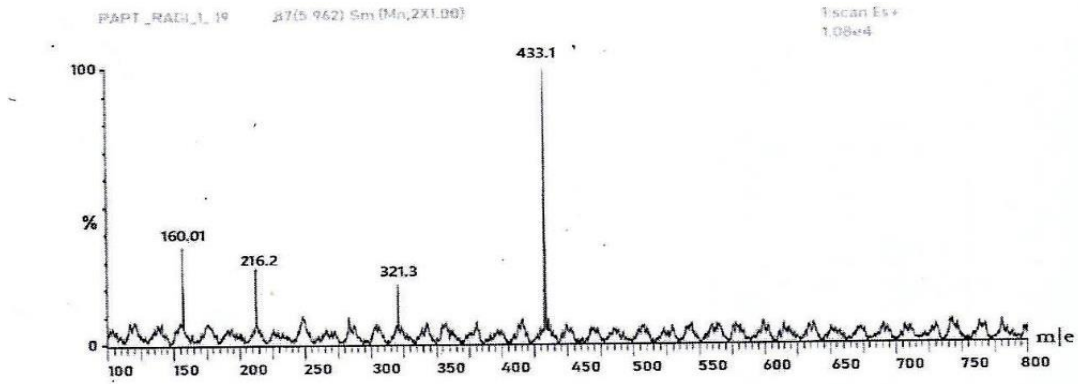


Figure 31: Mass Spectra of Isolated Compound

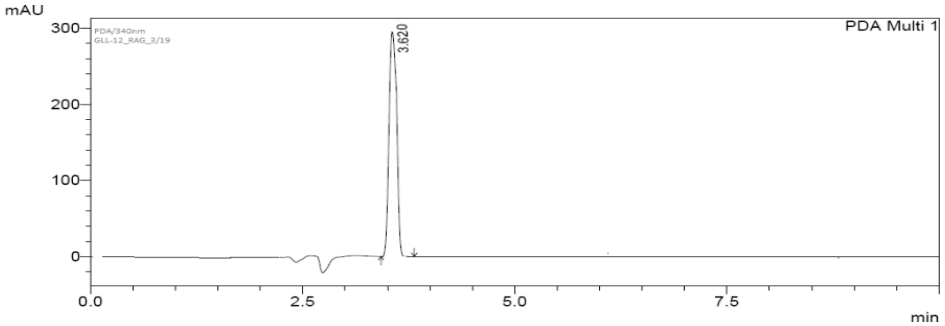


Figure 32: Liquid chromatography of isolated pure fraction Hydro-alcoholic extract of Glinus lotoides

The phytoconstituents present in Mollugo cerviana and Glinus Lotoides was separated by silica gel column chromatography.

A total of 44 50mL portions were taken & concentrated. TLC plates with concentrated fractions were used and stored in a chamber with mobile phase. The minor fractions were combined into 11 major fractions based on their TLC profiles. Table 5.22 shows the findings of the TLC profile. The first three minor fractions did not grow any spots, thus they were combined into one major fraction. Minor

fractions 5, 6, 7, and 8 all had one point with an Rf value of 0.73, which was collected as major fraction 2.

Minor fractions 9 to 12 had the same TLC profile as major fraction 3 and had Rf values of 0.63 and 0.88. Minor fractions 13,14,15,16 all have the same single place with an Rf value of 0.67, hence they were kept as major fractions. 4. The four minor fractions 17- 20 each have a single Rf value of 0.44 and are grouped together as major fraction 5. Minor

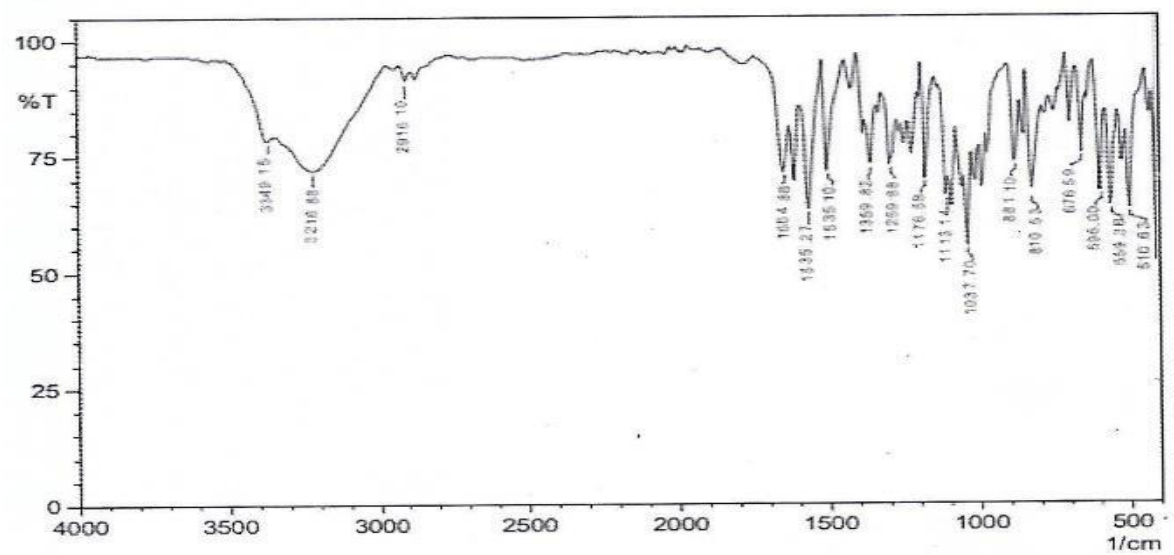


Figure 33: FTIR of Isolated compound II

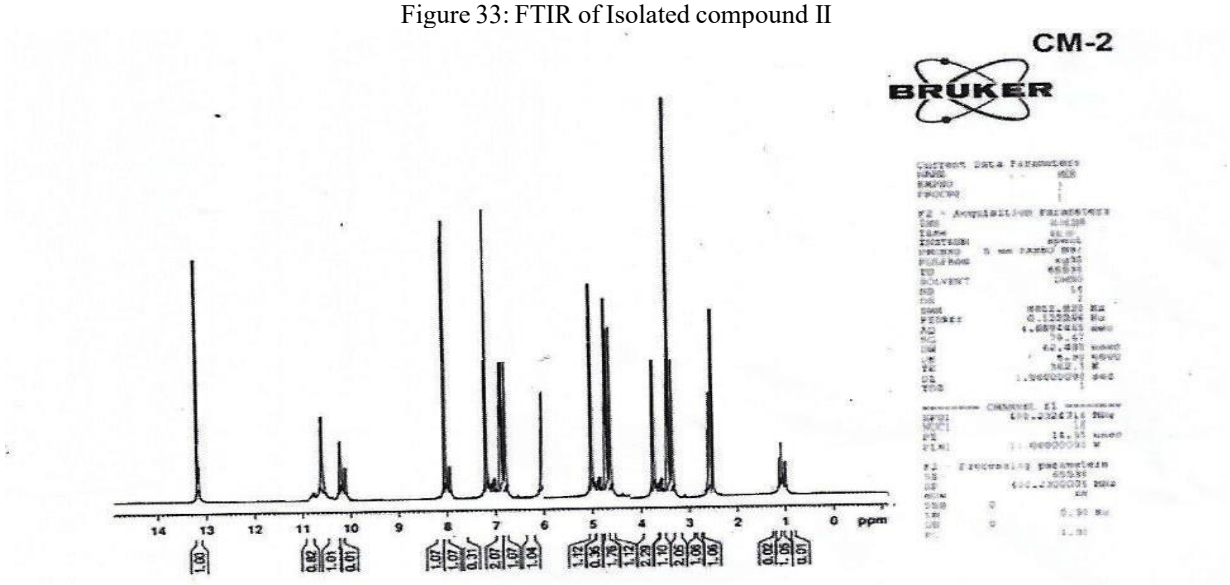


Figure 34: NMR spectra of isolated compound II

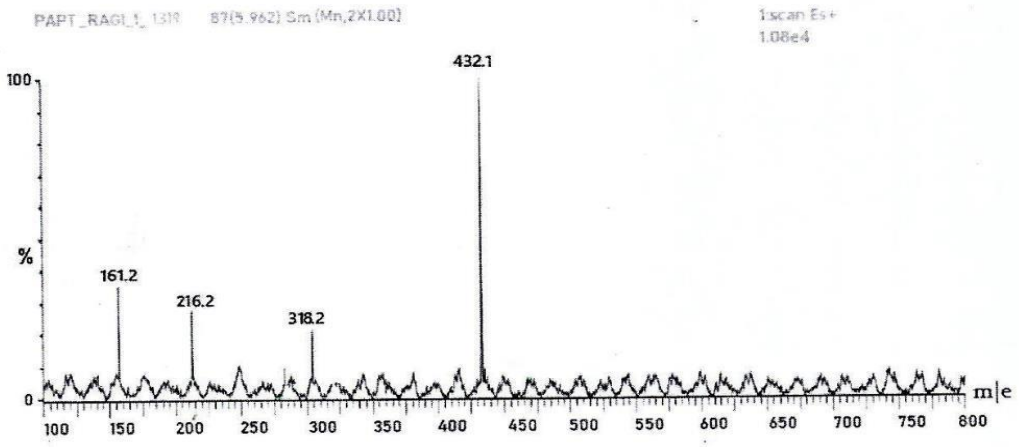


Figure 35: Mass Spectra of Isolated Compound II

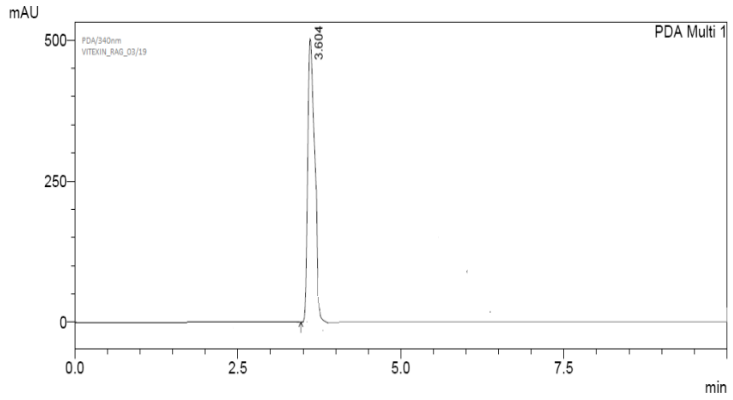


Figure 36: Chromatogram of vitexin standard

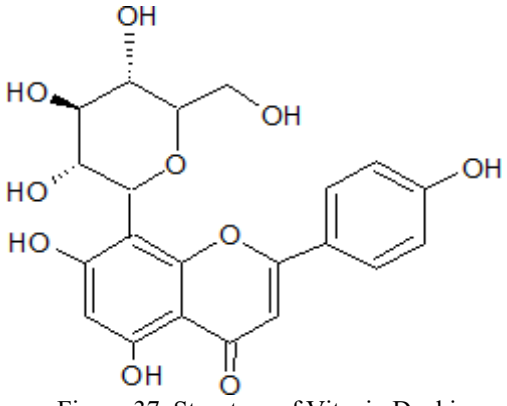


Figure 37: Structure of Vitexin Docking

fractions 21 to 24 had two places with 0.56 and 0.71, and the fractions were combined to form major fraction 6. The next four minor fractions 25, 28, and 29 reported no spot, hence they were dubbed major fraction 7. The TLC profile of the next four minor fractions, numbered 29 to 32, was the identical, with Rf 0.8 being designated as major fraction 8.

There were no spots in the four minor fractions from 33 to 36, hence they were kept as major fraction 9. Minor fractions 37 to 40 reported a single area with Rf 0.45 and were grouped together as major fraction 10. Minor fractions 41 to 44 have the same TLC profile with an Rf value of 0.88, so they were combined to form major fraction 11.

In vitro Antidiabetic Assay of Major Fractions

Seven and eight of the 11 major fractions collected and aggregated showed inhibitory activity on amylase, with 23.53 2.93 percent and 62.69 2.09 percent inhibitory activity, respectively (Table 5.18). When compared to the other major fractions, major fraction 8 had a larger percentage of inhibitory activity on amylase. As a result, this fraction is purified further using column chromatography and tested spectroscopically for the presence of an antidiabetic compound.

ISOLATED PURE FRACTION LIQUID CHROMATOGARPHY

A high-performance liquid chromatography was used to elute the identified chemical. The chromatogram that results displays a single peak at retention time 3.33. This demonstrates that the fraction contains a single chemical.

UV Spectrum of Isolated fraction I

The isolated portion was scanned in the UV range from 200 to 400 nm, with a clear peak at 205 nm. The presence of a single component in fraction No: 9 is demonstrated by the character of the spectrum and the maximum value. The UV Spectrum is depicted In Fig No: 5.30

The FTIR spectrum of Mollugo cerviana shows key peaks at 3349.1 cm^{-1} (glycosidic OH), 1635–1535 cm^{-1} (aromatic C=H stretch), 3216 cm^{-1} (O-H stretch), and 2916 cm^{-1} (C-H vibrations). Peaks at 1649.14–1107.70 cm^{-1} confirm aromatic structures, while 1037.70 cm^{-1} indicates an aromatic carbonyl group. The bands at 1382 and 1000 cm^{-1} correspond to C-O stretches. These spectral features confirm the presence of apigenin flavonoids in the ethanolic extract.

NMR Spectra of the Isolated Pure Compound

The isolated sample was identified as a light-yellow amorphous powder with an EI-MS m/z of [M+H] + 433.1 and a molecular formula of C12H20O10; 1H-NMR (DMSO- d6,400MHZ): The proton near the carbonyl group resonates at 13.10 (1H,s,H-23), the two protons near the aromatic ring resonate at 10.9 (1H,s,H-24) and 10.30(1H,s,H-25), and the rest of the protons resonate at 8.0 (2H,d,H-5,H-3), 7.9(1H,d,H-3), 6.9 (2H,d,H-2,H-6), 6.8 (1H,s,H-13), NMR was consistent with previous research on apigenin-8-C- beta-D-glucopyranoside after a thorough review (vitexin). Vitexin's molecular formula is C21H20O10.

Mass Spectra of Isolated Compound

The isolated compound's molecular ion peak (432.10 m/z) is similar to vitexin's mass. The obtained mass (432.86) differs somewhat from the expected mass due to the ionisation of the molecule (Fig. 5.13).

A total of 84 fractions (50 mL each) were concentrated and analysed using TLC. Based on TLC profiles, 18 major fractions were identified. Fractions without spots were combined. Notable RF values include 0.68 (fraction 2), 0.77 (fraction 4), 0.64 (fractions
5 & 6), 0.66 & 0.81 (fraction 7), 0.74 (fraction 8), 0.68 (fraction 10), 0.64 (fraction 12),
0.62, 0.87 & 0.79 (fraction 13), 0.78 (fraction 14), and 0.88 (fraction 16). Fractions 72– 82 showed three spots (0.68, 0.81, 0.76), while some fractions had no visible spots. Table 5.21 details the TLC findings**.**

In vitro Antidiabetic Assay of Major Fractions

Only one of the 18 significant portions (division No. 6) showed extensive inhibitory adequacy against - amylase (Table 5.22). Subsequently, this portion is refined further utilizing segment chromatography and tried spectroscopically for the presence of an antidiabetic compound.

Isolated pure fraction liquid chromatography

A high-performance liquid chromatography was used to elute the identified chemical. The chromatogram that results displays a single peak at retention time 3.33. The presence of a single chemical is thus confirmed in the fraction

FTIR of Isolated compound from Ethanolic extract of Glinus Lotoides

The frequency peaks at 3382.64 cm-1 indicate the existence of the OH group of the glycosidic ring, whereas the peak at 3137.27 cm-1 indicates the presence of the aromatic OH group. Glycosidic sp2 strech is indicated by the peak at 2917.65. (CH2). The presence of an aromatic carbonyl group in the isolated molecule may be seen in the

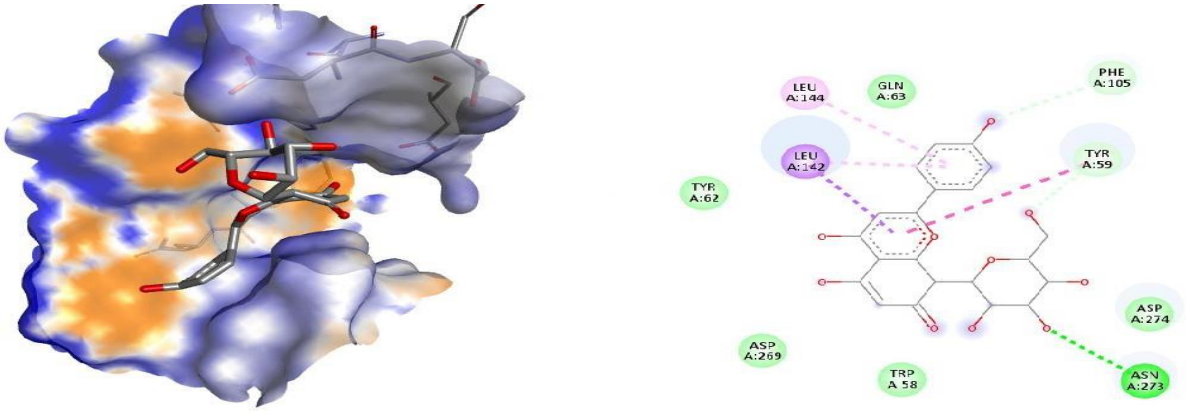


Figure 38: Binding of Amylase with Vitexin

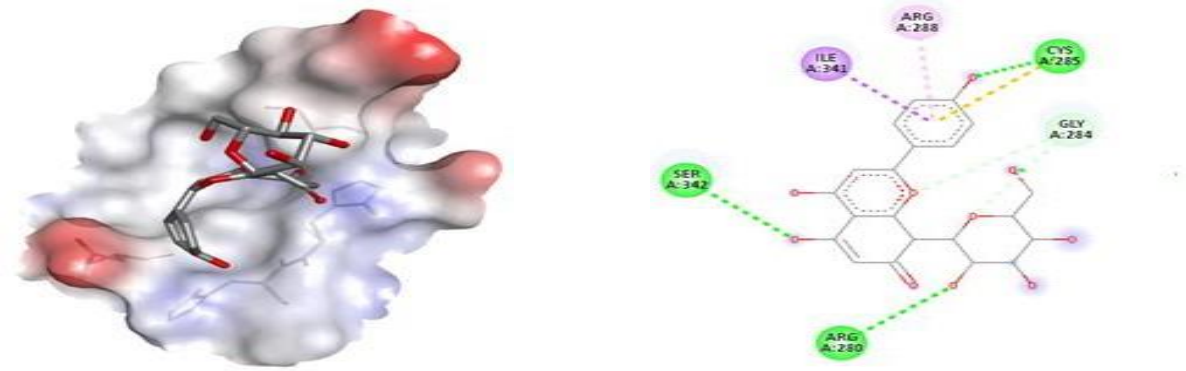


Figure 39: Alpaha glucosidase with Vitexin

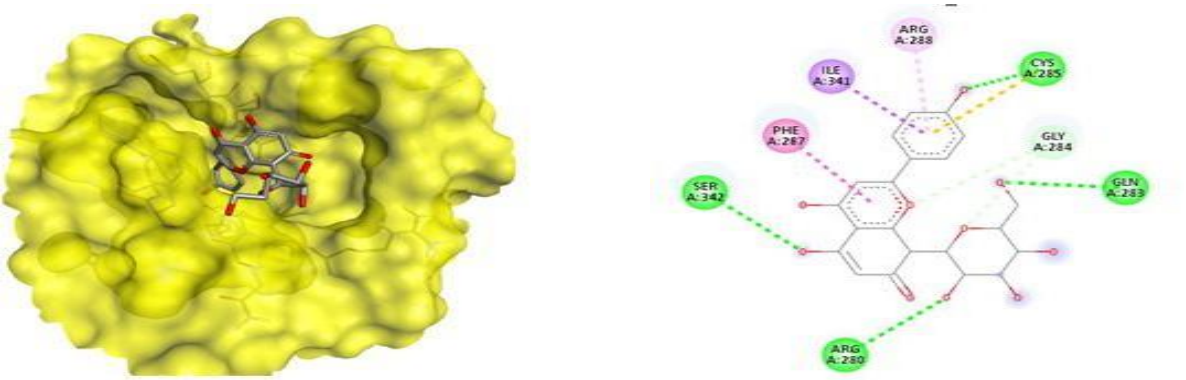


Figure 40: PPAR-Gamma with Vitexin

frequency peaks from 1649.14 to 1037.70 cm-1. In the hydroalcoholic extract of Glinus lotoides, the IR Spectrum confirms apigenin class of flavonoids.

NMR spectra of isolated compound from Hydro-alcoholic extract of Glinus lotoides

EI-MS m/z: [M+H] + 432.1, indicating a chemical formula of C12H20O10; 1H-NMR

(DMSO-d6,400MHZ): The resonance proton close to the carbonyl group is indicated by the peak at 13.40 (1H,s,H-23) All other protons resonate between 7.9 (2H,d,H-5,H- 3), 6.9 (2H,d,H-2,H-6), 6.7 (1H,s,H-13), 6.259 (1H,s,H-22), 5.0 (1H,s,H-26),

4.6(3H, m, H-27, H-28, H-29), 3.7 (2H, m, H-21), 3.6 (1H, d, H-16), 3.5 (2H, m, H-17)

Mass Spectra of Isolated Compound from Hydro-alcoholic extract of Glinus Lotoides

The molecular ion peak of the isolated compound is equal to the mass of the predicted component vitexin. Despite the fact that the obtained mass m/z (M +) 432.1 is identical to the expected mass, the fragment ion exhibits a minor deviation from the expected pattern.

Chromatogram of Vitexin Standard

The vitexin standard and leaf extricate from each test were weakened in methanol to a last convergence of 20 g/mL. The arrangements were sonicated for 5 minutes prior to being sifted with a 0.45 m breadth needle channel with HPLC investigation at 210 nm. The chromatogram of the vitexin reference standard.

Molecular docking analysis evaluated vitexin's antidiabetic potential by comparing its docking results with standard drugs acarbose and pioglitazone (Table 5.26). Docking was performed using AutoDock, with ligands treated as flexible molecules. The best docked conformations were selected based on the lowest energy values.

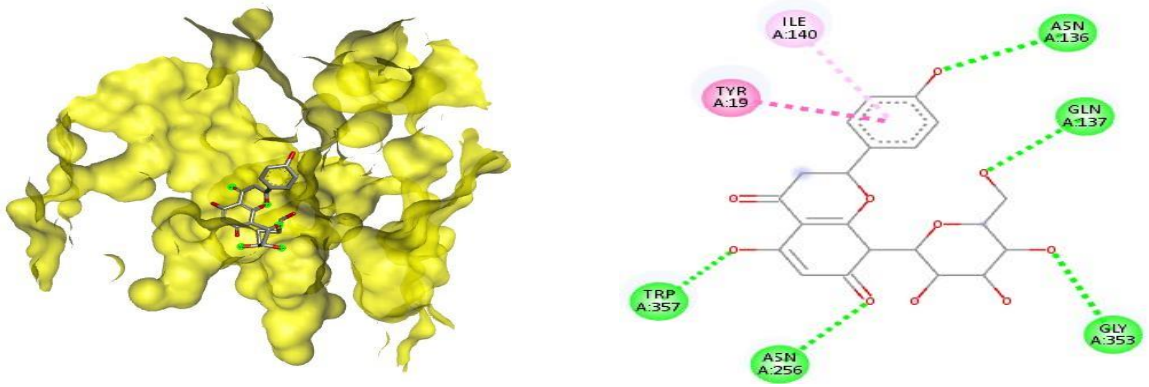


Figure 41: Glut 4 -Vitexin

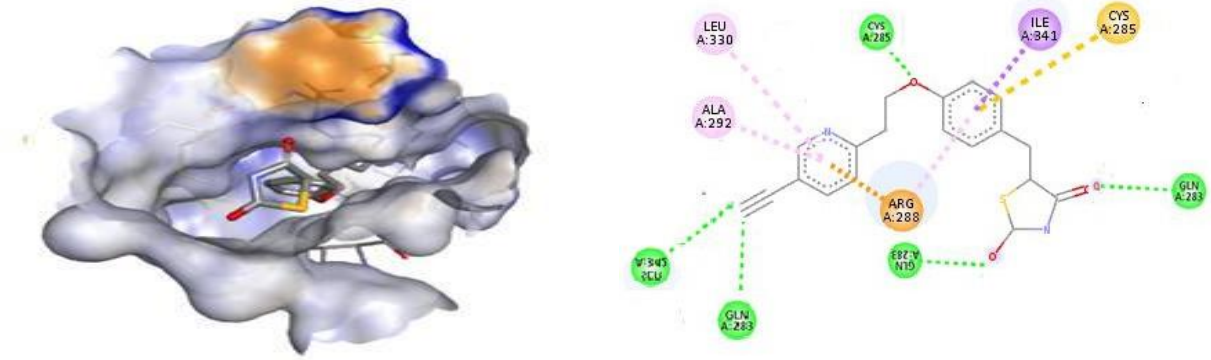


Figure 42: GLUT-4-pioglitazone

- **α-Amylase-vitexin**: -7.7 Kcal/mol (vs. acarbose: -8.7 Kcal/mol)
- **α-Glucosidase-vitexin**: -8.3 (vs. acarbose: -9.1)
- **GLUT-4-vitexin**: -9.5 (vs. pioglitazone: -9.3)
- **PPAR-vitexin**: -8.4 (vs. pioglitazone: -9.6)

Biovia Discovery Studio predicted docking models and hydrogen bonds, confirming vitexin's potential as an antidiabetic agent.

Vitexin's docking pattern closely matched pioglitazone, fitting the same active site in GLUT-4 with key amino acids like Arg 288, Ile 341, and Ser 342. Both vitexin and pioglitazone formed hydrogen bonds with these residues.

- **α-Amylase** showed a higher affinity for acarbose.
- **α-Glucosidase** preferred vitexin over acarbose.
- **GLUT-4 and PPAR** favored pioglitazone, but vitexin showed comparable binding.
- Vitexin had a stronger preference for **PPAR-γ and α-glucosidase**, suggesting its role in regulating these proteins in diabetes.

Following promising **in vitro** and **in silico** results, **in vivo** studies confirmed vitexin's antidiabetic potential from *Mollugo cerviana* and *Glinus lotoides*.

CONCLUSION

Diabetes treatment focuses on blood glucose control through diet and enzyme inhibition. α-glucosidase and α-amylase inhibitors help prevent post-meal blood sugar spikes. Acarbose, a common α-glucosidase inhibitor, has drawbacks, highlighting the need for new alternatives.

Indian Medicinal Plants & Anti-Diabetic Potential Mollugo cerviana and Glinus lotoides, members of the Molluginaceae family, are traditionally used for diabetes treatment. These plants, rich in flavonoids, were studied for their anti-diabetic effects. Ethanolic extract of *Mollugo cerviana* and hydro-alcoholic extract of *Glinus lotoides* were analyzed in vitro and in vivo, showing moderate to high anti-hyperglycemic activity.

Isolation & Docking Studies: The active compound Vitexin, a C-glycoside flavonoid, was isolated, purified, and characterized using chromatographic and spectrophotometric methods. Docking studies revealed that Vitexin enhances PPAR-γ and PPAR-α, improving insulin signaling and glucose regulation. By stimulating GLUT-4 expression, it helps in glucose uptake and reduces hyperglycemia.

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