A PROMISING ANTI-DIABETIC AGENT HAS BEEN ISOLATED FROM PREMNATOMENTOSA EXTRACT AFTER BEING STUDIED FOR IT'S POTENT ANTIOXIDANT EFFECTS

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Abstract:
Diabetes mellitus is a common chronic metabolic disorder characterized by elevated blood sugar levels and a high risk of complications. An isolated molecule from Premnatomentosa, apigenin glycoside, was studied in STZ-induced diabetic rats for its anti-diabetic properties and antioxidant mechanisms. Apigenin glycoside effectively controlled blood glucose levels, resulting in significant reductions on the 14th and 28th days. The treatment groups also improved various diabetes-related blood parameters, including plasma insulin levels, hemoglobin levels, HbA1c levels, total cholesterol levels, triglycerides, and HDL cholesterol levels. The findings suggest that apigenin glycoside can reduce cardiovascular risks and alleviate metabolic abnormalities. Furthermore, apigenin glycoside displayed potent antioxidant activity, as indicated by increased levels of SOD, CAT, and GPx enzymes, elevated levels of GSH, and decreased levels of MDA, which is a marker of lipid peroxidation. Apigenin glycoside is able to reduce oxidative stress and scavenge free radicals, as demonstrated by these results. As a result of the anti-diabetic and antioxidant effects observed in this study, Premnatomentosa and its bioactive constituents have become increasingly well-known for their therapeutic properties. Apigenin glycoside has the potential to be a promising therapeutic agent for managing diabetes and preventing complications associated with it, according to this study. In clinical settings, additional research is needed to elucidate the underlying molecular mechanisms and assess the long-term safety and efficacy of apigenin glycoside.

Keywords: diabetes mellitus, apigenin glycoside, Premnatomentosa, blood glucose, antioxidant, oxidative stress.

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INTRODUCTION

In recent years, diabetes mellitus, characterized by hyperglycemia, has reached epidemic proportions. A total of 463 million adults in 2019 were diagnosed with diabetes, and by 2045, this number will rise to 700 million (Federation, 2019), according to the International Diabetes Federation (IDF). Aside from its high prevalence, diabetes also exacerbates the quality of life of individuals affected by its complications, which significantly impact quality of life.

There are numerous complications associated with diabetes that affect multiple organ systems and increase morbidity and mortality. Chronic hyperglycemia, one of the hallmarks of diabetes, can cause microvascular and macrovascular complications (Riddle et al., 2019). There are several types of microvascular complications, including retinopathy, nephropathy, and neuropathy, whereas macrovascular complications include cardiovascular diseases such as coronary artery disease, strokes, and peripheral arterial diseases (Brownlee, 2005). Diabetes complications make it imperative to develop effective therapeutic interventions that can address not only glycemic control but also the underlying pathophysiological mechanisms that contribute to their development. The complex interplay of factors contributing to diabetes complications may not be fully addressed in traditional anti-diabetic medications that focus on glycemic management.

As a potential therapeutic agent for diabetes and its associated complications, natural compounds have gained increasing attention in recent years. Plant-derived compounds have shown promising pharmacological activities, including antioxidant and anti-inflammatory properties, which may reduce the detrimental effects of oxidative stress and inflammation in diabetes (Maritim et al., 2003). Traditionally used in several traditional medicine systems for treating various ailments, Premnatomentosa, known as Arani in Ayurveda, belongs to the Verbenaceae family and belongs to the family of Verbenaceae. A number of pharmacological properties are found in the plant (Dianita and Jantan, 2017), including anti-inflammatory, antioxidant, antidiabetic, antimicrobial, and hepatoprotective effects. In several studies, Premnatomentosa has been demonstrated to be antidiabetic, highlighting its antioxidant properties (Sridhar, 2013). As a continuation of the existing knowledge of the medicinal properties of Premnatomentosa, this study examined the potential therapeutic effects of a specific molecule isolated from its methanol extract on the management of diabetes and its complications. We specifically examined how the isolated molecule affected antioxidant parameters, which play a crucial role in mitigating oxidative stress, one of the major contributors to diabetes complications (Evans et al., 2002).

MATERIALS AND METHODS

Chemicals

All the chemicals and solvents used in the experiment were procured from SD fine chem LTD, India and are of analytical grade.

Plant material

Premnatomentosa leaves were collected from Tirumala hills in Andhra Pradesh. The plant parts have been certified by a certified botanist and a herbarium sample has been preserved for future reference. Shade dried leaves were harvested for 7 days and extracted with methanol using the Soxhlet apparatus. Using a rotary evaporator, the resulting extract (MPT) was concentrated (26.37% w/w) and stored at 4^{o}C. 8 fractions were isolated from the methanol extract using coloum chromatography and fraction 7 was subjected to coloum using dichloromethane and acetone (8:2). the isolated fraction 2 out of 5 was collected and subjected to further fractionation using methanol and chloroform (1:1). It yielded 2 fractions and the first fraction was collected and subjected to structural elucidation using TLC, HPTLC, FTIR and NMR and the resultant isolated molecule was identified as Apigenin-6,8-d-C-b-D-glucopyranoside. This is further purified and recrystallized and stored at 4oC till further use.

Experimental Animals

Healthy Wistar male albino rats (240-260 g) were maintained at a temperature of 25oC, a relative humidity of 50-50% with 12 h daylight and 12 h darkness. Drinking water was provided ad libitum, and they were fed a normal laboratory pellet diet. A seven-day acclimatization period was allowed for all animals before the experiment began. Animals were used under guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with approval of the Institutional Animal Ethics Committee.

Acute oral toxicity studies

Using adult Wister rats weighing 160 to 200 grams, the isolated compound and methanol extract (MEPT) was administered at an initial dose level of 2000 mg/kg body weight since most extracts had

LD50 values higher than 50 mg/kg p.o. Following overnight fasting, rats were administered doses ad libitum, while toxicity indicators were monitored for three to four hours after administration.

Antihyperglycemic Activity in Streptozotocin Induced Diabetic Rats

Animals were divided into 5 groups of six animals each.

Group I: rats (normal control) were treated with vehicle alone.

Group II: rats (disease control) were treated with vehicle alone

Group III: rats were treated with crude methanol extract (MEPT) (200mg/kg b.w).

Group IV: rats were treated with Isolated Molecule at 200 mg/ kg b.w. dose.

Group V: rats were treated with Glibenclamide (2.5 mg/kg b.w.).

Using carboxymethylcellulose (CMC) solution (0.5% w/v. in normal saline), streptozotocin (55 mg/kg b.w. in normal saline) was injected intraperitoneally (i.p.) once into overnight-fasted mice to cause type II diabetes (Prasad et al., 2009). The experimental group consisted of mice with elevated plasma glucose levels (200-300 mg/dL) 72 hours after STZ administration. Treatments were delivered once a day through a cannula for 28 days. Glucose levels were measured by a digital glucometer on days 0, 14 and 28 by drawing blood from a tail vein and measuring the blood glucose levels (Kind and King, 1954).

Estimation of enzymes, insulin and protein

On the 28th day, the animals had been starved for 12 hours, administered anesthesia, and then sacrificed by being beheaded. Blood samples were taken from an Eppendorf tube and left to coagulate for 30 minutes at room temperature. For the measurement of SGOT, SGPT, and ALP and liver glycogen, serum was centrifuged for 10 minutes at 2000 rpm (Reitman and Frankel, 1957; Plummer, 1978; Lowrry e al., 1951). The total protein was estimated using standard procedures (Paglia et al., 1965) using a radioimmunoassay kit from the Board of Radiation and Isotope Technology of Mumbai, India.

In-vivo Antioxidant Activity

On the 28th day, all animals were anesthetized with diethyl ether. On the following day, the livers were removed, dissected, and one portion was preserved in 10% formalin for histological examinations. An ELISA reader was used to measure malondialdehyde (MDA) production as a direct indicator of lipid peroxidation (LPO). Additionally, tris-HCl buffer was used to homogenize the rest of the liver for the determination of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx)activities(Noor et al., 2008).

Histopathological studies

After the 28th day, the animals were fasted for 12 hours, anaesthetized with diethyl ether, and sacrificed by cervical dislocation. An ice-cold saline solution was immediately used to dissect out, excrete and rinse the pancreas. Observations of histopathology were conducted under a light microscope (40x) after fixing, dehydrating, and staining with haematoxineosin (Kasetti et al., 2010).

Statistical analysis

The data were analyzed by using GraphPad Prism 5.0 (San Diego, USA). The data were analyzed using one-way ANOVA followed by Dunnett's test for statistical significance differences between the biochemical estimations and the negative control ($p<0.05$).

RESULTS

Acute Oral Toxicity studies

During acute toxicity tests, rats at a dose of 2000 mg/kg did not experience any adverse effects from the isolated molecule. The rats were not significantly affected by haematological or biochemical indicators (liver enzymes and serum lipids) in comparison with vehicle-treated rats. In this case, the dose was 10% of the highest dose used in the acute toxicity study, or 200 mg/kg, and further research was undertaken.

Table 1: Acute toxicity studies of the isolated molecule

S. No.	Observation Days	Control	MEPT (2000mg/kg)	Isolated Molecule (2000mg/kg)
1	Baseline	194.87±0.99	190.75±2.31	193.75±2.76
2	3rd day	197.37±1.92	193.37±1.99	201.62±2.87
3	6th day	198.25±1.48	195.12±1.24	197.37±2.44
4	9th day	205.5±1.85	207.37±1.84	207±1.85
5	14th day	208.37±3.06	207.37±1.76	212.12±2.23

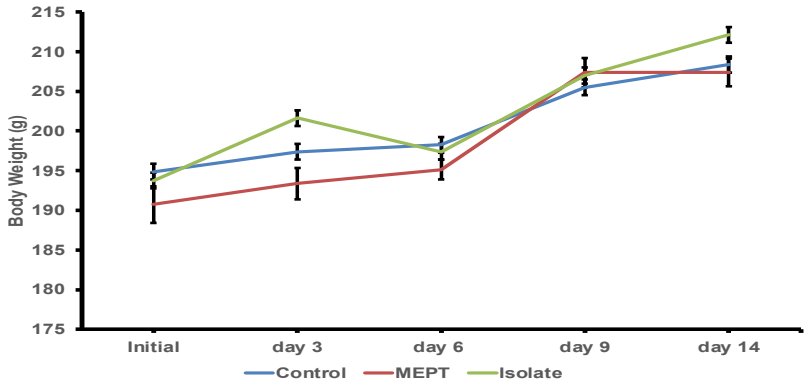

Figure 1: Changes in the body weight of rats administered with isolated molecule

Effect of Isolated molecule on the STZ induced diabetes

The table 2 provides a comprehensive summary of the body weight measurements obtained from a research study investigating the anti-diabetic potential of a molecule isolated from the methanol extract of Premntatomentosa. There were no adverse effects or external factors that influenced the weight changes of the normal control, indicating a stable body weight throughout the entire study. It was found that the diabetic control had a significant reduction in weight both after 14 days and after 28 days. The decline is consistent with the expected consequences of uncontrolled diabetes, which often results in weight loss due to metabolic imbalances. A methanol extract of Premnatomentosa was administered to the test group (MEPT 200mg/kg). In contrast to the control group, this group did not show any significant changes in body weight, suggesting the extract may have potential therapeutic properties for managing diabetes and did not adversely affect weight. In group 4, apigenin glycoside (200mg/kg) was isolated and used to treat diabetes. In both cases, the body weight of group 4 exceeded the weight of the diabetic control and treatment control groups. This study indicates that the isolated molecule is effective at regulating weight and may have an anti-diabetic effect that is more potent. Group 4 treated with the isolated molecule also showed improvements in body weight, comparable to those observed in the standard treatment group.

Table 2: Effect of Isolated molecule on the body weight of STZ induced diabetic rats

GROUPS	TREATMENT	BODY WEIGHT		
		0 DAY	14 DAY	28 DAY
Group 1	Normal Control	210.5±1.87	225.83±1.60	243.5±2.07
Group 2	Diabetic Control	209.33±1.50	196.83±1.72^{*a}	173.5±3.27^{*a}
Group 3	Treatment Control (MEPT-200mg/kg)	212.83±1.94	229.83±2.22^{*b}	242.5±1.87^{*b}
Group 4	Isolate-200mg/kg	216±3.52	235.5±2.58^{*b}	251±2.09^{*b}
Group 5	Standard (Glibenclamide 2.5 mg/kg)	212.33±2.87	239.33±2.25^{*b}	252.83±2.48^{*b}

The data is represented as Mean±SEM; n=6; *p<0.001 is considered significant when compared to a=control group, b=diabetic control

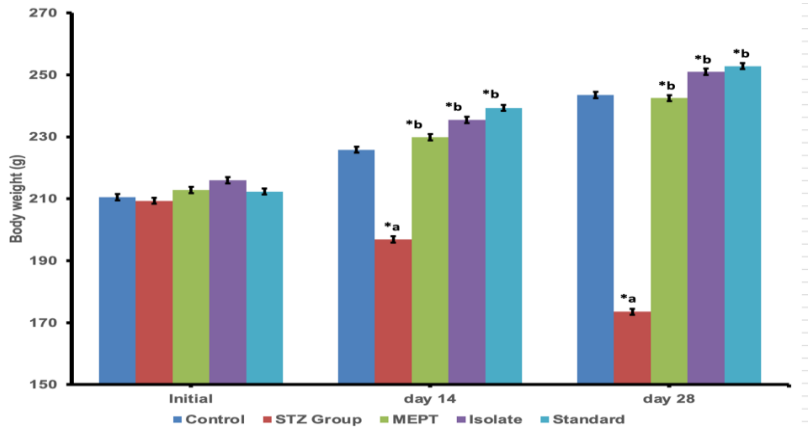

Figure 2: Effect of the isolated molecule on the STZ induced Diabetic rats

The blood glucose level remained consistently low throughout the study with normal control. It is given in table 3 that the blood glucose level at 0 day was 92,193 mg/dL, followed by an increase of 93,880 mg/dL at 14 days and 96,190 mg/dL at 28 days. Compared to the normal control group, diabetic control demonstrated significantly elevated blood glucose levels at all time points. At 0 day, the blood glucose level was measured at 264.84 mg/dL. As the blood glucose level increased over the course of 14 and 28 days, it reached 285.29 mg/dL and 303.41 mg/dL, respectively. These values show uncontrolled hyperglycemia associated with diabetes. The blood glucose levels of Group 3 initially were higher than that of the normal control group. However, at 14 and 28 days, blood glucose levels were significantly reduced. At 14 days, the blood glucose level was measured at 187.11 mg/dL, and at 28 days, it dropped to 168.50 mg/dL. As a result, the methanol extract has the potential to manage hyperglycemia and reduce blood glucose levels. Both the diabetic control group and the treatment control group had a significant decrease in blood glucose levels in groups 4 and 5. The blood glucose level of the patients decreased to 145.64 to 132.22 mg/dL after 14 days, and to 169.62 and 145.9 mg/dL after 28 days, respectively. These values indicate the efficacy of the isolated molecule in lowering blood glucose levels and potentially serving as an anti-diabetic agent.

Table 3: Effect of Isolated molecule on the blood glucose level of diabetic rats

GROUPS	TREATMENT	BLOOD GLUCOSE LEVEL (mg/dL)		
		0 DAY	14 DAY	28 DAY
Group 1	Normal Control	92.193±1.59	93.880±2.90	96.190±2.42
Group 2	Diabetic Control	264.84±3.29	285.29±3.20^{*a}	303.41±3.07^{*a}
Group 3	Treatment Control (MEPT-200mg/kg)	273.73±4.78	187.11±3.87^{*b}	168.50±5.15^{*b}
Group 4	Isolate-200mg/kg	267.18±3.02	169.62±4.13^{*b}	145.64±3.05^{*b}
Group 5	Standard (Glibenclamide 2.5 mg/kg)	264.46±2.87	145.90±2.25^{*b}	132.22±2.48^{*b}

The data is represented as Mean±SEM; n=6; *p<0.001 is considered significant when compared to a=control group, b=diabetic control

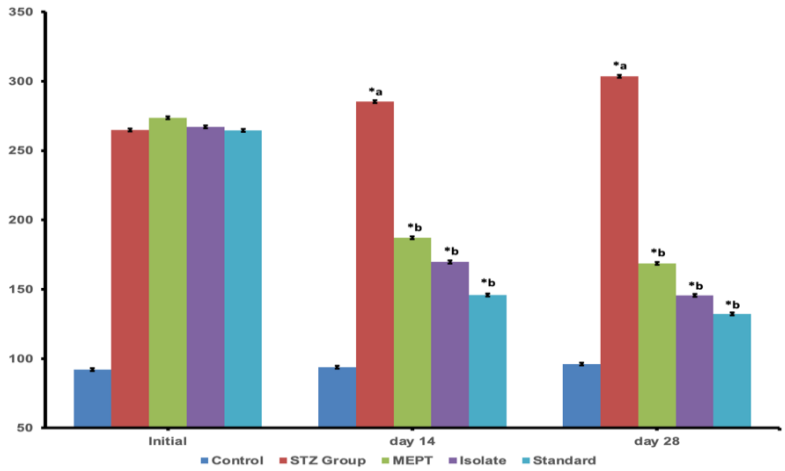

Figure 3: Effect of isolated molecule on the blood glucose levels of STZ induced Diabetic rats

As shown in Table 4, the isolated molecule affected various blood parameters in streptozotocin (STZ) induced diabetic rats. Plasma insulin levels were observed at 18.93 mL/ml in the Normal Control group on the 28th day. Hemoglobin (HB), glycated hemoglobin (HBA1C), total proteins, cholesterol, triglycerides, and high-density lipoprotein (HDL) levels were measured at 12.47 mg/dl, 6.497%, 7.826 g/dl, 145.20 mg/dl, 80.69 mg/dl, and 46.06 mg/dl, respectively. Diabetes control group plasma insulin levels decreased to 5.531 mL/mL, significantly lower than normal control group levels. There were also significant changes in levels of HB, HBA1C, total protein, total cholesterol, triglycerides, and HDL, reflecting metabolic dysregulation associated with diabetes. Compared to the diabetic control group, MEPT treatment improved several blood parameters. Levels of HB, HBA1C, total proteins, total cholesterol, triglycerides, and HDL also improved. Plasma insulin increased to 12.38 l/ml, while total proteins, total cholesterol, triglycerides, and HDL also improved. Plasma insulin levels increased to 14.38 g/dL, and HB, HBA1C, proteins, cholesterol, triglycerides, and HDL levels controlled similar to those in the Standard group.

Table 4: Effect of isolated molecule on the blood parameters of STZ induced diabetic rats

GROUPS	Plasma Insulin (µLitre/ml) 28 th day	HB(mg/dl)	HBA1C (%)	Total Proteins(g/dl)	Total cholesterol(mg/dl)	Triglyceride (mg/dl)	HDL(mg/dl)
Normal Control	18.93±0.39	12.47±0.58	6.497±0.27	7.826±0.30	145.20±2.01	80.69±1.95	46.06±1.38
Diabetic Control	5.531±0.40*a	8.106±0.34*a	13.81±1.33*a	4.777±0.24*a	271.40±4.58*a	173.4±2.41*a	24.40±1.23*a
Treatment Control (MEPT-200mg/kg)	12.38±0.26*b	12.44±0.25*b	8.897±0.24*b	6.107±0.53*b	193.59±3.20*b	123.4±1.37*b	29.10±1.83*b
Isolate-200mg/kg	14.38±0.95*b	12.70±0.99*b	7.849±0.33*b	7.198±0.43*b	184.45±3.36*b	98.04±2.66*b	34.70±1.36*b
Standard (Glibenclamide 2.5 mg/kg)	17.07±1.01*b	12.98±0.39*b	7.105±0.62*b	7.782±0.50*b	170.41±2.94*b	88.11±2.67*b	37.10±1.00*b

The data is represented as Mean±SEM; n=6; *p<0.001 is considered significant when compared to a=control group, b=diabetic control

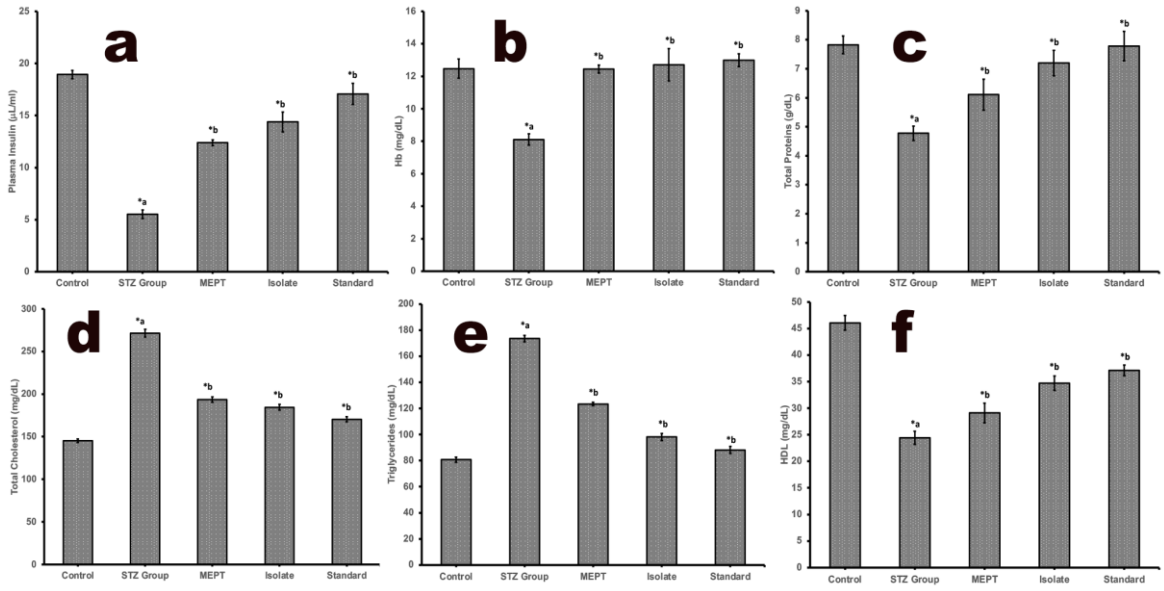

Figure 4: Effect of the isolated molecule on the blood parameters a. Plasma Insulin, b. Hb, c. Total Proteins, d. Total Cholesterol, e. Triglycerides, f. HDL

As shown in Table 5, the isolated molecule affected liver and kidney parameters in diabetic rats induced by streptozotocin (STZ). The levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea, and creatinine in the Normal Control group were 26.45 U/L, 45.37 U/L, 121.2 U/L, 30.92 mg/dL, and 1.03 mg/dL, respectively. A significant difference was observed between the Diabetic Control group and the normal control group in these liver and kidney parameters. ALT, AST, ALP, urea, and creatinine levels were measured at 55.23 U/L, 91.79 U/L, 241.7 U/L, 81.25 mg/dL, and 2.66 mg/dL, respectively. In comparison with the diabetic control group, the MEPT group showed improvements in liver and kidney parameters. A positive change was seen in the levels of ALT, AST, ALP, urea, and creatinine. Similarly to the Standard group, the Isolate group also improved liver and kidney parameters.

Table 5: effect of isolated molecule on the liver and kidney parameters in STZ induced diabetic rats

GROUPS	ALT(U/L)	AST(U/L)	ALP(U/L)	UREA(MG/DL)	CREATININE (MG/DL)
Normal Control	26.45±0.98	45.37±1.50	121.2±3.69	30.92±1.58	1.03±0.17
Diabetic Control	55.23±2.08*a	91.79±1.42*a	241.7±3.08*a	81.25±2.30*a	2.66±0.28*a
Treatment Control (MEPT-200mg/kg)	47.01±1.45*b	66.86±0.92*b	183.7±2.05*b	55.90±2.12*b	1.34±0.38*b
Isolate-200mg/kg	37.70±1.11*b	57.51±0.96*b	163.6±2.65*b	49.20±0.86*b	1.32±0.14*b
Standard (Glibenclamide 2.5 mg/kg)	30.05±1.49*b	49.97±0.64*b	148.9±1.12*b	40.20±1.20*b	1.03±0.12*b

The data is represented as Mean±SEM; n=6; *p<0.001 is considered significant when compared to a=control group, b=diabetic control

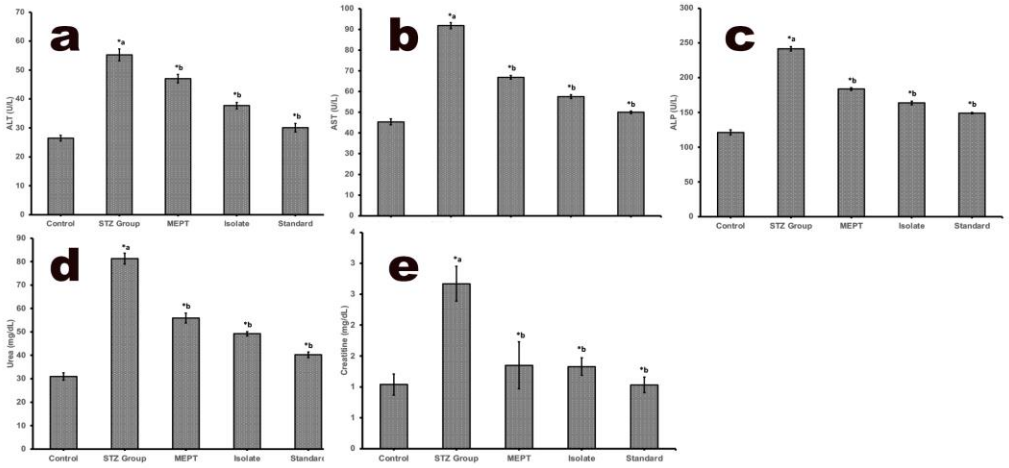

Figure 5: Effect of Isolated molecule on the Liver and kidney paramters of the STZ induced diabetic rats a. ALT, b. AST, c. ALP, d. Urea and e. Creatinine

As shown in Table 6, the isolated molecule and the standard anti-diabetic medication significantly reduced antioxidant parameters in streptozotocin (STZ) induced diabetic rats. The antioxidant parameters of the Diabetic Control group, such as SOD, CAT, GPx, GSH, and MDA, were significantly altered compared to the Normal Control group, which indicates increased oxidative stress associated with diabetes. The antioxidant parameters of the Diabetic Control group, such as SOD, CAT, GPx, GSH, and MDA, were significantly altered compared to the Normal Control group, which indicates increased oxidative stress associated with diabetes. In comparison with the diabetic control group, apigenin glycoside demonstrated significant improvements in antioxidant parameters. This suggests the molecule has potent antioxidant properties compared to the standard drug, Glibenclamide, based on the positive changes in the levels of SOD, CAT, GPx, GSH, and MDA.

Table 6: Effect of isolated molecule on the antioxidant parameters of the STZ induced diabetic rats

GROUPS	SOD Unit/mg Protein	CAT Mmol/min/mg Protein	GPx Mmol/min/mg Protein	GSH Mm/100mg Tissue	MDA Mmol/100mg tissue
Normal Control	9.46±0.53	95.85±2.12	10.07±0.26	56.81±2.12	6.05±0.37
Diabetic Control	4.48±0.36^{*a}	43.12±2.21^{*a}	5.40±0.28^{*a}	23.76±2.63^{*a}	12.4±0.29^{*a}
Treatment Control (MEPT-200mg/kg)	6.92±0.28^{*b}	55.20±1.06^{*b}	7.87±0.25^{*b}	40.77±2.30^{*b}	6.54±0.64^{*b}
Isolate-200mg/kg	7.39±0.31^{*b}	65.12±2.24^{*b}	8.91±0.31^{*b}	46.58±1.68^{*b}	5.41±0.35
Standard (Glibenclamide 2.5 mg/kg)	8.58±0.20^{*b}	84.99±2.17^{*b}	9.69±0.25^{*b}	51.76±1.26^{*b}	2.48±0.27^{*b}

The data is represented as Mean±SEM; n=6; *p<0.001 is considered significant when compared to a=control group, b=diabetic control

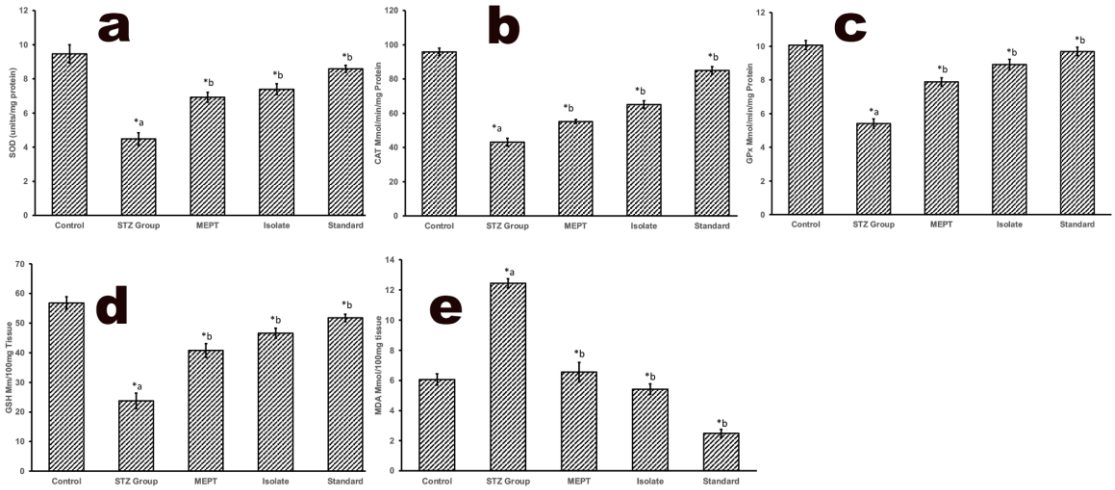

Figure 6: Effect of Isolated molecule on the antioxidant parameters of the STZ induced diabetic rats a. SOD, b. CAT, c. GPx, d. GSH, e. MDA

Histopathological observations

The pancreas of diabetic rats treated with STZ showed widespread necrotic alterations, damaged cell populations, and reduced islet size. These changes were followed by fibrosis and atrophy (B). In rats treated with an isolated molecule (Apigenin glycoside) and metformin, necrotic and fibrotic alterations were repaired, and they had increased their number and size of islets (C). It was observed that normal acini and cellular structures were found in the pancreatic islets of Langerhans of the normal control group (A). As with AG-treated rats, Glibenclamide-treated rats (D) showed changes in pancreatic morphology. A diagram attached illustrates how 5HAG affects the pancreatic region in both normal rats and diabetic rats.

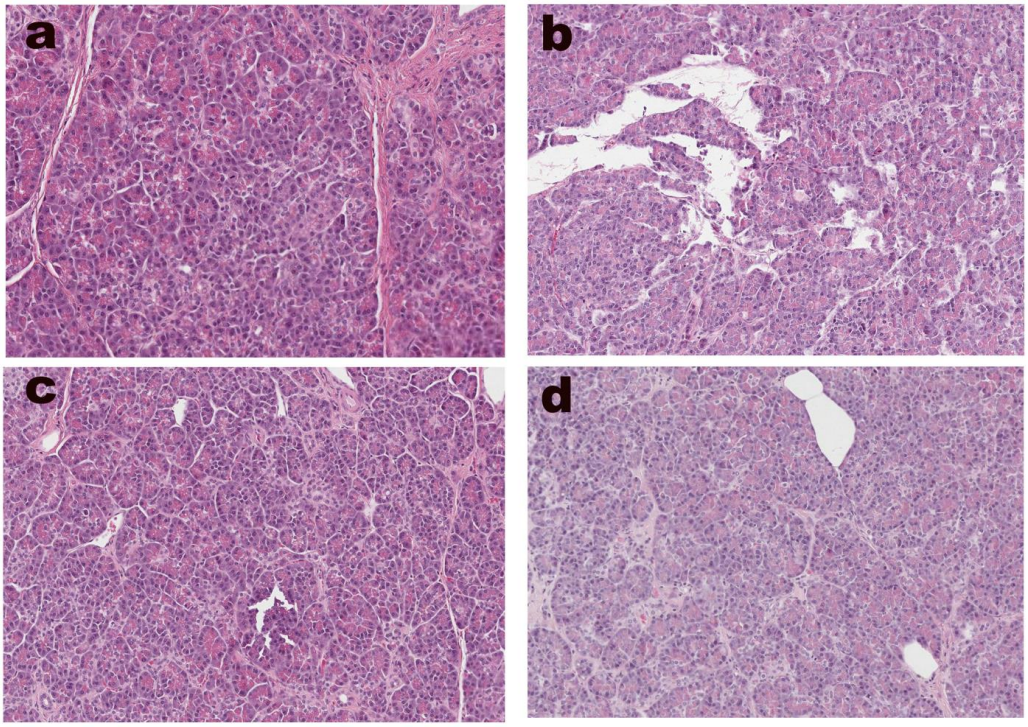

Figure 7: Effect of Isolated apigenin glycoside on the histology of Pancreas of STZ induced diabetic ratsa. normal group, b. disease control, c. Isolated apigenin, d. Standard drug

DISCUSSION

This study evaluated the anti-diabetic potential of apigenin glycoside, an isolated molecule extracted from Premnatomentosa in methanol. STZ-induced diabetic rats demonstrated significant effects of apigenin glycoside on controlling diabetes and related complications. Additionally, it provided insight into the antioxidant mechanisms responsible for its therapeutic effects. As compared with the diabetic control group, the treatment groups also demonstrated a significant decrease in blood glucose levels at the 14th and 28th days after treatment. In addition to enhancing insulin secretion, apigenin glycoside could also improve insulin sensitivity (Gowtham et al., 2018; Bnouham et al., 2006; chao et al., 2018).

Additionally, apigenin glycoside had significant effects on various blood parameters related to diabetes complications, in addition to controlling glycemia. When compared to the diabetic control group, the treatment groups showed favorable changes in plasma insulin levels, hemoglobin (HB), HbA1c, total proteins, total cholesterol, triglycerides, and HDL cholesterol. As a result of these findings, apigenin glycoside may be effective in treating diabetes-related metabolic abnormalities and reducing other complications. One of the noteworthy aspects of the study is the exploration of the antioxidant parameters influenced by apigenin glycoside treatment. Increased oxidative stress in diabetes leads to tissue damage and diabetic complications (Rains and Jain, 2011). It was found that the treatment groups had significant improvements in antioxidant enzyme activities, such as SOD, CAT, GPx, and GSH, as well as a reduction in levels of MDA. Based on these findings, it appears that apigenin glycoside exerts its protective effects by scavenging free radicals and preventing oxidative damage with its potent antioxidant properties.

Earlier studies on Premnatomentosa and other related compounds have shown that apigenin

glycoside is effective in controlling diabetes and its complications. Premnatomentosa extracts have been shown to have anti-diabetic effects, attributed to apigenin glycoside, one of its bioactive constituents. In addition, apigenin glycoside has been extensively studied for its antioxidant properties, highlighting its role in preventing oxidative stress-related diseases (Singh et al., 2004).

CONCLUSION

It is clear from this study that apigenin glycoside isolated from Premnatomentosa has significant anti-diabetic effects. As well as its antioxidant properties, the findings demonstrate its ability to effectively control blood glucose levels and improve diabetic parameters. Apigenin glycoside is a potential therapeutic agent for managing diabetes and its associated complications, based on these findings. In order to determine its long-term safety and efficacy, further investigations are needed to elucidate its precise molecular mechanisms.

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