Therapeutic role of kaempferol and myricetin in streptozotocin induced diabetes synergistically via modulation in pancreatic amylase, glycogen storage and insulin secretion

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Abstract

Aim

Kaempferol and Myricetin alone have promising benefits on diabetes and related complications, yet the effectiveness of cotreating the two compounds on diabetes have not been studied. The existing investigation was to study the combined anti-diabetic effect of kaempferol and myricetin in streptozotocin (STZ)-activated diabetes in rats.

Methods

To evaluate the antidiabetic activity, 36 Wistar rats were segregated into six groups; Normal, 50 mg/kg STZ-induced diabetes, and four (50 mg/kg kaempferol, 50 mg/kg myricetin, 25mg/kg kaempferol + myricetin, and 5mg/kg glibenclamide) compound-treated diabetic groups. The effects of co-treatment on parameters, glucose, insulin, lipid profile, liver enzymes, antioxidant biomarkers, and inflammatory cytokines were measured.

Results

The study revealed that combined treatment restored the assessed parameters including glucose levels, insulin levels, inflammatory cytokines, oxidative markers, and lipid and liver enzymes in diabetic rats.

Conclusion

The results indicate that cotreatment of kaempferol and myricetin has beneficial role against diabetes suggesting that cotreatment of these compounds can be used therapeutically in treating diabetes.

1. Intrduction

Diabetes, a chronic metabolic condition is classified into types: Type I which is caused due to insufficient pancreatic production of insulin, and Type II diabetes which develops due to impaired glucose secretion [12]. In total, 10% of cases are of type I diabetes and 90% populated affected by type II diabetes [3]. The affected population has been found with visible symptoms such as hunger, intense thirst, frequent urination, unusual weight loss/weight gain, bruises and cuts which are difficult to heal, fatigue, tingling, and numbness in feet and hands, sexual dysfunction in male [4]. Diabetic patients have low antioxidant markers because of increased oxidative cell injury and tissue damage [5]. Long-term hyperglycemia may damage and fail the vital organs including kidney, heart, eyes, and blood vessels which may lead to complicated disorders such as diabetic neuropathy/ nephropathy, cardiovascular diseases, and retinopathy [6 7].

Streptozotocin (STZ), a nitrosourea analogue is cytotoxic for pancreatic ß-cell causing a hyperglycaemic condition in rats. Exposure of STZ methylates the DNA proteins which ultimately is responsible for ß-cell death and functional deficits [8]. STZ is a highly selective agent when administered as a single dose cause ß-cell necrosis and induction of diabetes within 48hrs [9]. It targets the GLUT2 receptors which are present in ß-cells and also in the liver and kidney, so the destruction of ß-cell impairs the liver and kidney functions [10]. Thus, the STZ model has been chosen for the induction of diabetes in rats.

Diabetes is currently treated with oral Hypoglycaemic drugs which may help in regulating the glucose levels but have undesirable long-term side effects [11]. So, exploration towards natural therapeutic agents is an alternative option for the management of diabetes and also has comparatively less/ no side effects. Traditional herbs have a tremendous medicinal value which is because of the phytochemicals present exerting their actions. Kaempferol is a bioactive flavonoid, present in several plants and foods derived from such as beans, kale, broccoli, spinach, and tea. Kaempferol is known to have a potential antioxidant, anti-inflammatory, and antidiabetic activity [12 13]. Kaempferol also reduced the oxidative damage produced by STZ, although weak absorption, high metabolic rate, and a fast systemic elimination restrict the pharmacological potency of the compound [14].

Myricetin is a polyphenol compound that belongs to the flavonoid family, with prominent antioxidant potential. It is obtained from common food sources which include tomatoes, oranges and other fruits, tea, berries, and nuts. Myricetin also possesses several pharmacological actions including antioxidant, anti-inflammatory, hypoglycaemic ability [15 16].

Kaempferol and myricetin have a very strong traditional background for effectiveness against diabetes and related complications. It has been shown that Kaempferol and myricetin together work synergically and have increased the antioxidant potential compared to alone [17]. So, the current study was aimed to examine the combined effect of kaempferol and myricetin in STZ generated diabetes.

2. Materials And Methods

2.1 Chemicals

Glibenclamide, kaempferol, myricetin, STZ and other chemicals were purchased from local vendor and were of standard analytical range.

2.2 Animals

Adult, healthy, male Wistar rats 6–7 weeks-old weighing around 150-200g were procured. The rats were separated into groups and kept in standard cages under controlled environment with the temperature set at (22˚C ± 3˚C), relative humidity 60–75% and light (14:10 h dark and light cycle). The animals were fed with pellet diet with surplus amount of water. The research study was permitted by the Institutional Animal Ethical Committee (IAEC- TRS/PT/021/009) followed under the direction of the CPCSEA.

2.3 Initiation of diabetes in rats

STZ was used for inducing diabetes in rats. The animals were starved for 15 h before to the induction time. STZ was freshly made in citrate buffer which was intraperitoneally (i.p.) injected at dose of 50 mg/kg b.wt [18 19]. After injection, the animals received glucose (5%) overnight to control drug-related hypoglycaemia. The induction of diabetes was checked by recording the glucose level 72 h after STZ injection and then treatment was started for the next 28 days.

2.4 Study protocol

A total of 36 rats were randomly acclimatized-allocated in different groups of six (n = 6) for 28 days. (Fig. 1)

Group-I: Normal Control group (NC), wistar rats received 10ml/kg 0.1% sodium carboxymethylcellulose (SCMC) per oral (p.o) for 28days.

Group-II: Diabetes Control (DC), STZ was injected as intraperitoneal dose of 50 mg/kg b.wt and then continued receiving 10ml/kg/p.o SCMC for 28 days.

Group-III: STZ- activated animals received 50 mg/kg p.o kaempferol daily for 28 days [20].

Group-IV: STZ- activated animals received 50 mg/kg p.o myricetin daily for 28 days [21].

Group-V: STZ- induced animals received kaempferol and myricetin 25 mg/kg p.o daily for 28 days.

Group-VI: STZ- induced animals received oral dose 5mg/kg Standard Glibenclamide daily for 28 days [22 23].

After induction with STZ, the animals displayed diabetes-like symptoms such as polyuria, dyspepsia, bodyweight within 48hrs. The blood glucose was measured after 72hrs of STZ injection. The rats which showed a glucose level of more than 250mg/dl were confirmed diabetic and further preferred for treatment. On 29th day, blood was collected from all the groups and euthanized under high anaesthesia. The liver and pancreas were collected immediately and stored under formalin solution (10%) for biochemical estimation.

2.5 Measurement of bodyweight

The animals were recorded for basal bodyweight (start of the study) and final bodyweight at the completion of the study before euthanization.

2.6 Measurement of blood glucose

The glucose levels were collected by tail vein puncture and a drop was placed on one- time glucose strip by using an electronic ACCU-CHEK glucometer on day 0, 7th, 14th, 21st, and 29th day [24].

2.7 Estimation of serum insulin

The serum insulin level was estimated by commercial Rat ELISA insulin kit as per the standard provided method in the assay protocol.

2.8 Estimation of serum amylase

Serum amylase activity was estimated colorimetric method by using 3,5-dinitrosalicylic acid as a substrate.

2.9 Determination of hepatic glycogen

Liver glycogen was measured by following anthrone method [25]. The homogenate was prepared by homogenizing liver tissue in 1 mL of 5% potassium hydroxide which was boiled for 35 min. Then, 5 mL ethanol was added for glycogen precipitation. The tubes were centrifugated for 10 mins. Finally, after addition of anthrone reagent produced green colour which was recorded at 620 nm.

2.10 Determination of lipid levels and liver markers

The collected blood was centrifugated at 2000 × g for 20 mins and the supernatant was separated for further estimation. The lipid levels such as total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) were assessed spectrophotometrically by following standard assay protocols. The protein was estimated by performing the biuret colorimetric method [26].

The biomarkers for liver include aspartate transaminase (AST), alanine transaminase (ALT), which were assessed by standard assay kits. The enzymes serve as a biomarker for hepatic injury that determines the enzyme concentration that draws into the systemic circulation.

2.11 Biochemical estimation

Preparation of homogenate

The pancreas was excised, collected, washed with cold saline, and then homogenized in a homogenizing vessel at 700 rpm with 0.1M tris-HCL to obtain 10% w/w homogenate. The homogenate was centrifugated at 2000 × g for 15 mins at 4˚C. The separated layer was further utilized for the estimation of antioxidant markers.

2.11.1 Estimation of Superoxide dismutase (SOD)

The reaction mixture contained 0.5ml homogenate sample, ethanol (2ml), and chloroform (1ml, the tubes were shaken and centrifugated. The mixture included 1 ml sodium pyrophosphate buffer, 0.1ml N-methyl dibenzo pyrazine methyl sulphate, 0.3ml nitroblue tetrazolium (NBT), 0.2ml reduced nicotinamide adenine dinucleotide (NADH) and were incubated for 10 mins, and the reaction was ceased by adding of glacial acetic acid. The mixture was mixed by adding n-butanol. The chromogen was measured

spectroscopically at 560nm. One enzyme unit is defined as 50% inhibition of NBT reduction produced in 1 minute. The activity was presented as unit/min/mg of protein [27 28].

2.11.2 Determination of reduced glutathione (GSH)

The procedure was performed by following the method given by the Ellman method. The reaction tube contained 1ml homogenate sample and 2ml trichloroacetic acid (TCA) which was centrifugated to collect the supernatant. To the supernatant, 1ml Ellman's reagent and disodium hydrogen phosphate were mixed. The colour produced was measured by a UV spectrophotometer at 412nm. The GSH activity was presented as µmol/mg protein for tissues [29 30].

2.11.3 Determination of lipid peroxidation (MDA)

To the supernatant, 0.2ml sodium lauryl sulphate, 1.5ml acetic acid, 1ml thiobarbituric acid (TBAR) was added and heated for upto 1hr. On cooling 5ml of n-butanol and pyridine was mixed into the enzyme solution. The mixture was then centrifugated at 4000rpm for 15mins. The absorbance was measured by separating the organic phase at 532nm spectrophotometrically. The MDA activity was presented as nM MDA/mg protein of tissue [31 32].

2.11.4 Determination of Catalase (CAT)

Catalase assay was based on the decomposition of hydrogen peroxide by catalase enzyme. To 0.5ml sample solution, potassium phosphate (50mM), and 0.1ml hydrogen peroxide (H_2O_2) in phosphate buffer (50mM) were added. The H_2O_2 decomposition was recorded at 240nm at 15- sec intervals. The activity was calculated as nmol/mg protein of tissue [33 34].

2.12 Determination of inflammatory cytokines

The level of IL-6, IL-1β, and TNF-α were quantified by immunoassay kit. The separated protein was used to coat the antibody ELISA wells. The amount of enzyme marker was determined by following the assay procedure.

2.13 Statistical analysis

The results were presented as mean ± SEM. The data were estimated by using both one-way following Tukey's comparison test and two-way ANOVA following Bonferroni's test using Graph pad prism software. One-way ANOVA was used for calculating the difference between the two groups by setting criteria of significance at $P < 0.05$.

3. Results

Change in body weight

The basal body weight of rats before starting the study was compared with the body weight which was measured on the 29th day. The STZ-induced group showed a marked decrease in the body weight ($P < 0.001$) while the treatment groups i.e., kaempferol and myricetin treated groups showed a marked amelioration ($P < 0.01$) and combined treatment (kaempferol and myricetin) showed a significance of $P < 0.001$. (Fig. 2)

Effect on blood sugar level

The blood glucose level remarkably raised ($P < 0.001$) from day 7th to 21st in STZ group. While on day 29th, the blood glucose was markedly reduced ($P < 0.05$) in all treatment groups including kaempferol, myricetin, combined kaempferol + myricetin group and glibenclamide treated group. (Fig. 2)

Effect on serum insulin level

The diabetic group displayed a noticeable decline ($p < 0.001$) in the insulin levels when correlated to controls. When treated with kaempferol and myricetin group, a significant rise in the insulin level was observed having $P < 0.05$ significance while in kaempferol + myricetin group and glibenclamide treated group also elevated the insulin level ($P < 0.01$) when correlated to the diabetics. (Fig. 3)

Effect on serum amylase

The STZ group displayed a marked elevation ($P < 0.001$) in the amylase activity compared to the controls. The treatment groups i.e., kaempferol and myricetin -treated rats ($P < 0.05$), co-treatment group ($P < 0.01$), and glibenclamide ($P < 0.001$) displayed a marked decline in the amylase activity when correlated to the diabetic group. (Fig. 4)

Effect on glycogen levels

The STZ group showed a considerable rise ($P < 0.001$) in the glycogen level when correlated to the controls. The treatment groups i.e., kaempferol and myricetin -treated rats ($P < 0.01$), kaempferol + myricetin- treated rats ($P < 0.001$), and glibenclamide ($P < 0.001$) displayed a pronounced reduction in the glycogen level when correlated to the diabetic controls. (Fig. 5)

Effect on lipid profile and liver enzymes

A remarkable increase ($P < 0.001$) in the TG and triglyceride levels and decline in the HDL and total protein was observed in STZ group when correlated to the controls. The treatment groups i.e., kaempferol and myricetin rats ($P < 0.01$), kaempferol + myricetin - treated rats ($P < 0.01$) and std glibenclamide ($P < 0.001$) showed a marked decline in the cholesterol and triglyceride level. While a considerable rise in the HDL and total protein was examined in treatment groups i.e., kaempferol and myricetin- treated rats ($P < 0.05$), kaempferol + myricetin - treated rats ($P < 0.05$, $P < 0.01$) and glibenclamide ($P < 0.05$ and $P < 0.01$) respectively as correlated to the diabetic controls. (Fig. 6)

Both AST and ALT were markedly elevated ($P < 0.001$) in diabetic group as correlated to the normal rats. Treatment groups: kaempferol and myricetin -treated rats ($P < 0.05$), kaempferol + myricetin rats ($P < 0.001$) and glibenclamide ($P < 0.001$) remarkably decreased the liver enzymes in comparison to diabetic group. (Fig. 7)

Effect on antioxidant enzymes

The STZ group showed a marked decline ($P < 0.001$) in the CAT, SOD, and GSH levels as correlated to the controls. The treatment groups i.e., kaempferol and myricetin -treated rats ($P < 0.01$ and $P < 0.05$), kaempferol + myricetin - treated rats ($P < 0.001$, $P < 0.01$ and $P < 0.05$) and glibenclamide ($P < 0.001$, $P < 0.001$ and $P < 0.05$) showed a marked decline in the CAT, SOD and GSH activity respectively when correlated to the diabetic controls. The MDA level was markedly elevated in the STZ group when corelated to the normal while the treatment groups i.e., kaempferol -treated rats ($P < 0.01$), myricetin - treated rats ($P < 0.001$), kaempferol + myricetin - treated rats ($P < 0.001$), and std glibenclamide ($P < 0.001$) displayed a marked downfall in the MDA levels in comparison to the diabetic rats. (Fig. 8)

Effect on pro-inflammatory cytokines

The diabetic group presented a remarkable rise ($P < 0.001$) in the IL-6, IL-1β, and TNF-α when correlated to the normal control. The treatment groups displayed a sharp decline in the IL-6, IL-1β, and TNF-α levels especially the cotreatment group (kaempferol + myricetin) in comparison to the diabetic controls. (Fig. 9)

4. Discussion

Hyperglycemia involves excessive production in hepatic glycogenolysis and gluconeogenesis and depletion in the utilization of tissue glucose which leads to cause hepatic and renal failure [35 36]. The increasing threat of diabetes leads to the search of medicinal plants which are more efficacious and safer for long-term use. Among them, kaempferol and myricetin are natural phytochemicals belonging to the flavonoid group. They possess pharmacological actions, including anti-oxidant, anti-inflammatory, neuroprotective, antidiabetic properties [37 38].

Individual treatment of kaempferol and myricetin have shown curative effects on type-II diabetes in vivo by improving glucose level, antioxidant, lipid, and liver enzymes [39 40]. Both kaempferol and myricetin work synergistically when cotreated and their combined treatment has not been studied in diabetics. So, the current study focuses on the anti-diabetic actions of kaempferol and myricetin and its cotreatment using STZ model in rats.

We examined whether kaempferol and myricetin alone or in combination could decrease the glucose, glycogen, and insulin levels in diabetic controls. Previous findings displayed that STZ elevated the glucose level, reduced the glycogen and plasma insulin levels in rats which were in line with the current study [41 42]. The current investigation revealed that co-treatment with kaempferol and myricetin normalized the glucose level, increased the glycogen and insulin levels near to normal compared to

treatment with kaempferol and myricetin alone indicating cotreatment helped in the restoration of destructed pancreatic ß-cells. Serum amylase activity was significantly increased in STZ-induced diabetes which was in-line with the previous studies [43]. Treatment with kaempferol and myricetin markedly ameliorated the level of amylase, which confirmed better glycaemic control by cotreatment compared to treatment alone.

Diabetic dyslipidemia is a risk aspect for related complications which includes cardiovascular disorders, nephrotoxicity, atherosclerosis. Earlier studies found that STZ increased cholesterol, triglyceride, and reduced HDL and protein levels which were similarly observed in current study [44 45]. Our investigation displayed that kaempferol and myricetin treatment lowered the serum lipid levels (TC, TG) and elevated the HDL levels. Co-treatment with kaempferol and myricetin effectively enhanced these levels than treatment with one of the compounds did alone.

The liver plays a major role in glycogenolysis but in diabetic conditions, the liver conversion increases leading to overproduction of glucose causing hyperglycemia [46]. The study indicated that the glucose regulating hepatic enzymes were impaired in STZ-induced rats which was in-line with the previous studies [47 48]. The treatment displayed a marked decrease in the liver enzymes (AST, ALT) especially cotreatment with kaempferol and myricetin. The hypoglycaemic activity of drugs in diabetic rats was may be due to the restoration of liver markers.

Oxidative stress results in increased tissue lipid peroxidation leading to overproduction of free radicals and inactivation of membrane-bound antioxidant enzymes. Previous investigations were in line with the present study which showed that STZ-induced group suppressed the antioxidant enzymes causing deficiency [49 50]. Cotreatment with kaempferol and myricetin effectively restored the antioxidant enzymes (SOD, CAT, GSH, and MDA) than treated alone.

Long-term innate system activation may lead to inflammation resulting in diabetic complications and disease progression. Overproduction of cytokines cause functional damage to the tissues. Consistent with the findings, our study showed increased cytokine production in diabetic rats [51 52]. While cotreatment with kaempferol and myricetin in diabetic rats significantly decreased the cytokine level near to standard glibenclamide when correlated to the alone treated group.

Conclusion

In conclusion, the investigation revealed that kaempferol, myricetin, and their combination have the potentiality to repair hyperglycemia, glucose dysfunction, oxidative stress, inflammatory cytokines, and dyslipidemia in STZ-activated diabetes. Our study proved that cotreatment with kaempferol and myricetin at a dose of 25mg/kg can be therapeutically used for the management of diabetes.

Declarations

Contradictions

F.A.A.-A. designed, interpreted and wrote manuscript. I.K. performed work.

Compliance with ethical standards

Declaration of competing interest

The author reports no conflict of interest

Ethical Approval

The research study was permitted by the Institutional Animal Ethical Committee (IAEC- TRS/PT/021/009) followed under the direction of the CPCSEA.

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Figures

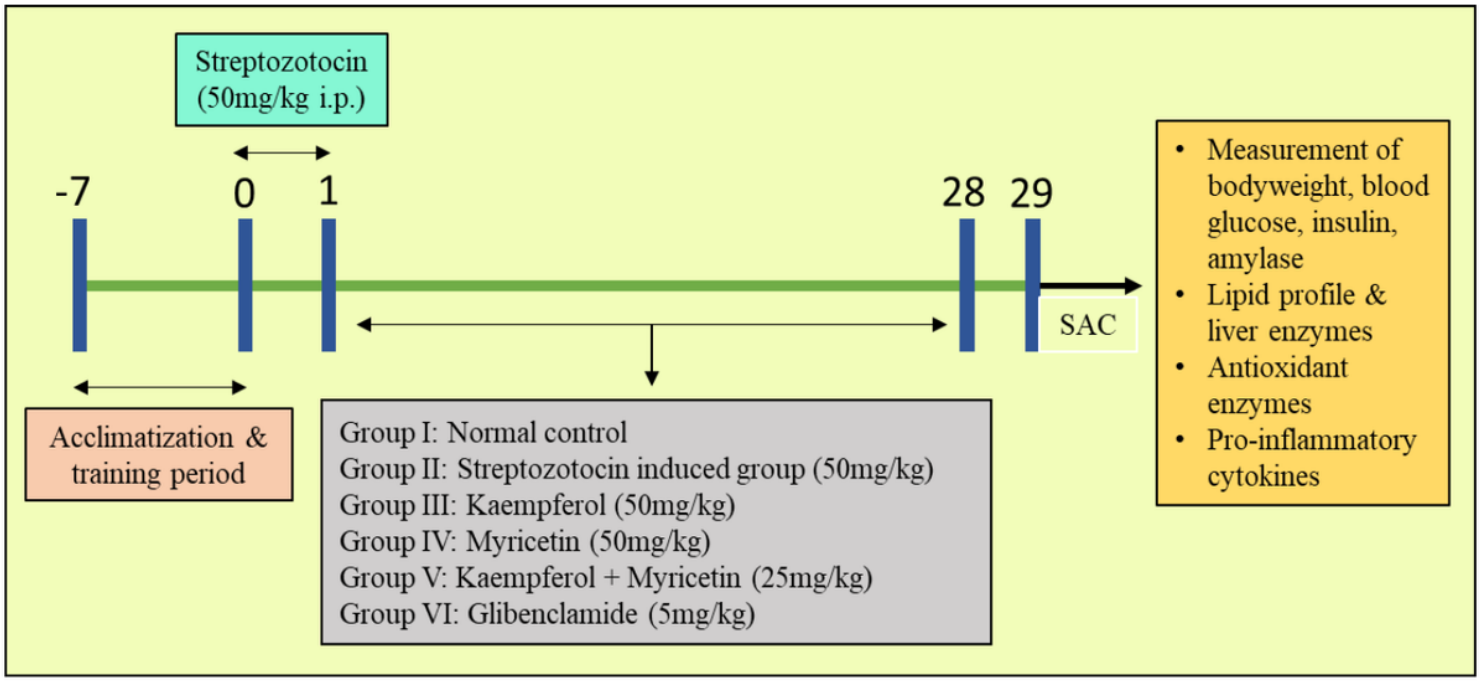

Figure 1

Experimental protocol

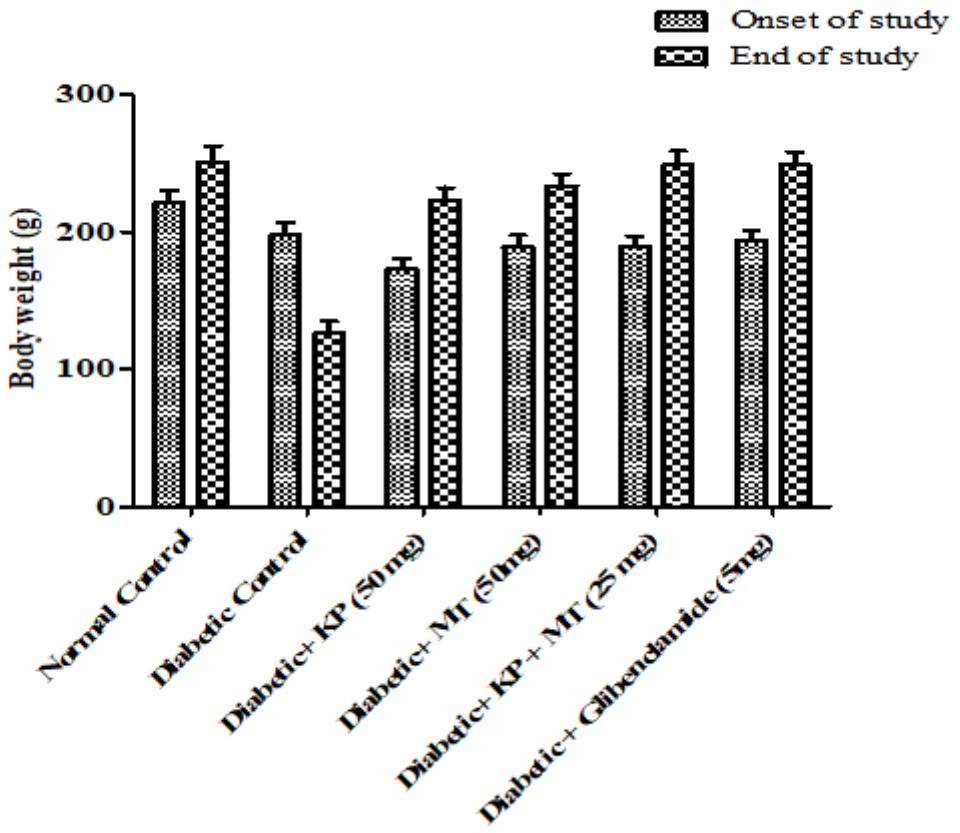

Figure 2

Effect of kaempferol and myricetin on bodyweight in streptozotocin-induced rats. All values are oresented as mean ± SEM. Corelation amid the groups was done using Bonferroni post comparison test by two-way ANOVA.

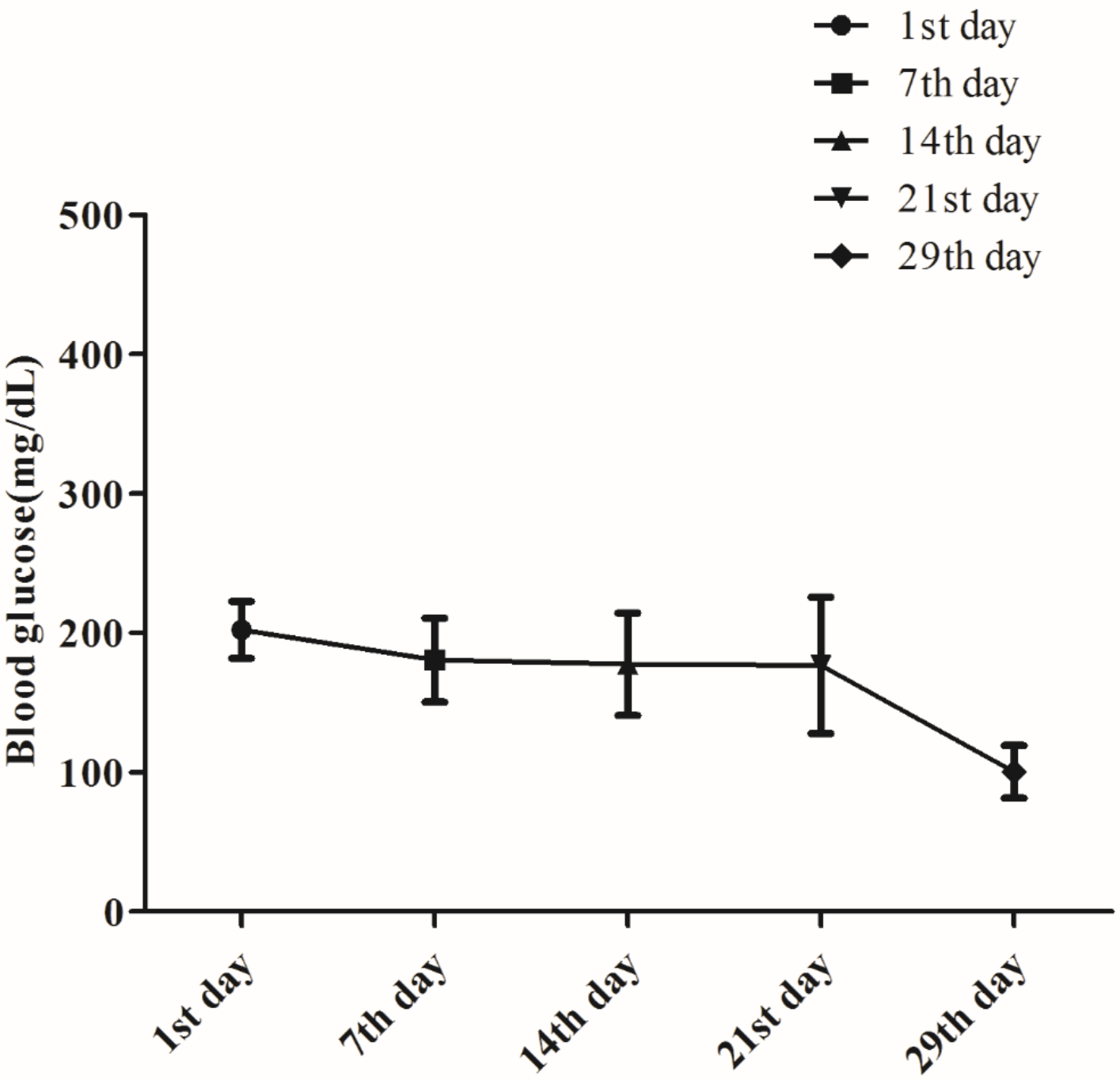

Figure 3

Effect of kaempferol and myricetin on glucose in streptozotocin-induced rats. All values are presented as mean ± SEM. Corelation amongst the groups was done using Bonferroni post comparison test by two-way ANOVA.

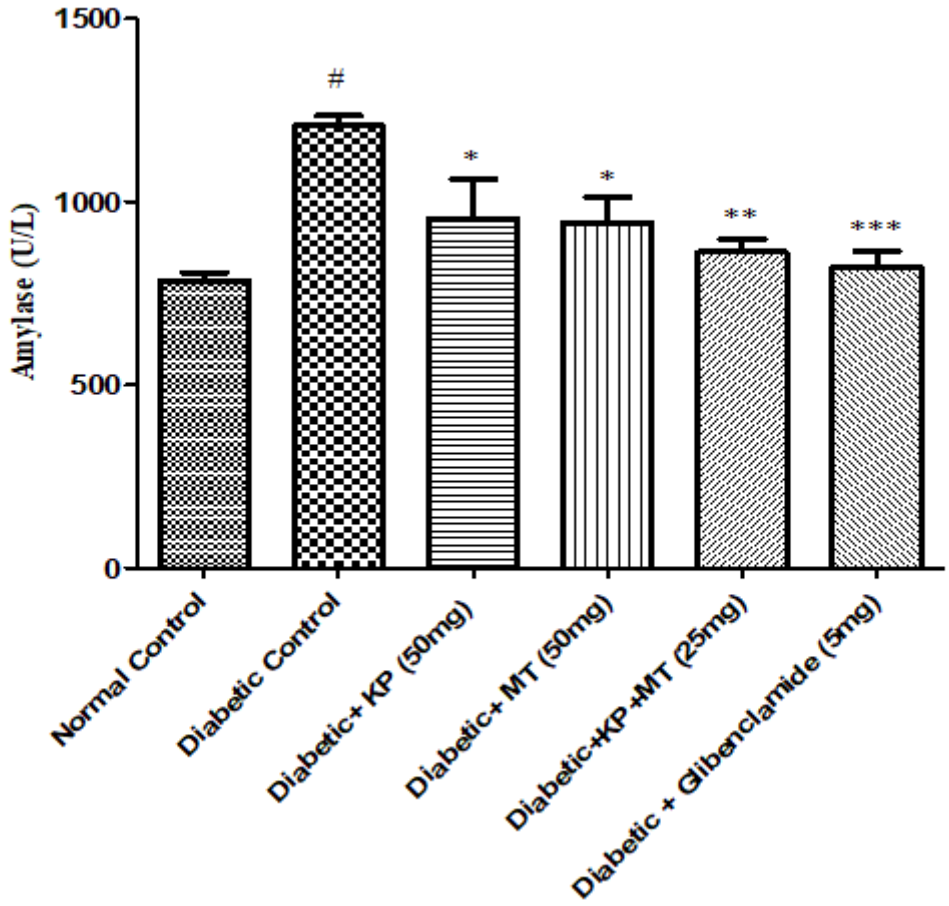

Figure 4

Effect of kaempferol and myricetin on amylase in streptozotocin-activated rats. All values were indicated as mean ± SEM. Corelation amid the groups was done using Tukey's post comparison test by one- way ANOVA.

P value < 0.5, 0.01, 0.001 was expressed as *, **, *** when correlated to diabetic group

Signifies comparison to control group (P<0.001)

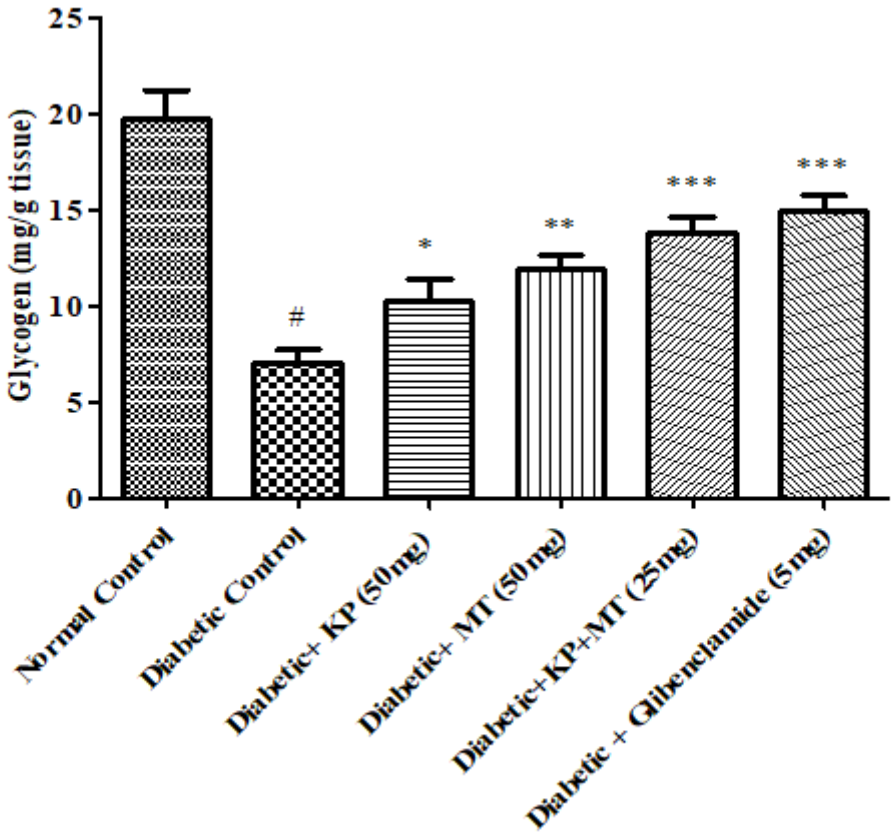

Figure 5

Effect of kaempferol and myricetin on amylase in streptozotocin-induced rats. All values are presented as mean ± SEM. Corelation amid the groups was done using Tukey's post comparison test by one- way ANOVA.

P value < 0.5, 0.01, 0.001 was expressed as *, **, *** when correlated to diabetic group

Signifies correlation with control group (P<0.001)

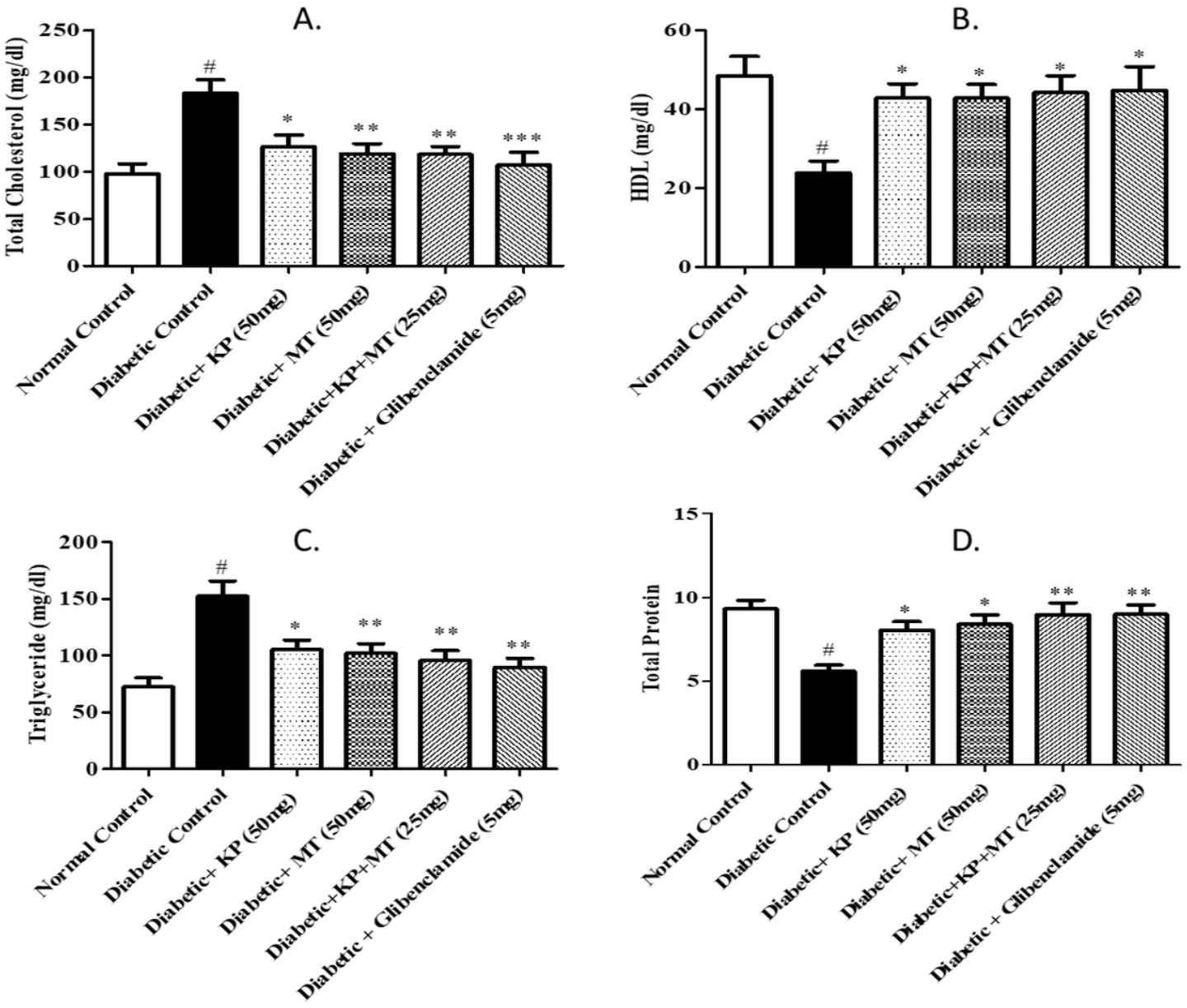

Figure 6

Effect of kaempferol and myricetin on lipid profile A. Total cholesterol, B. HDL, C. Triglycerides, and D. Total protein in streptozotocin-induced rats. All values are presented as mean ± SEM. Corelation amid the groups was done using Tukey's post comparison test by one- way ANOVA.

P value < 0.5, 0.01, 0.001 was expressed as *, **, *** when correlated to diabetic group

Signifies correlation with control group (P<0.001)

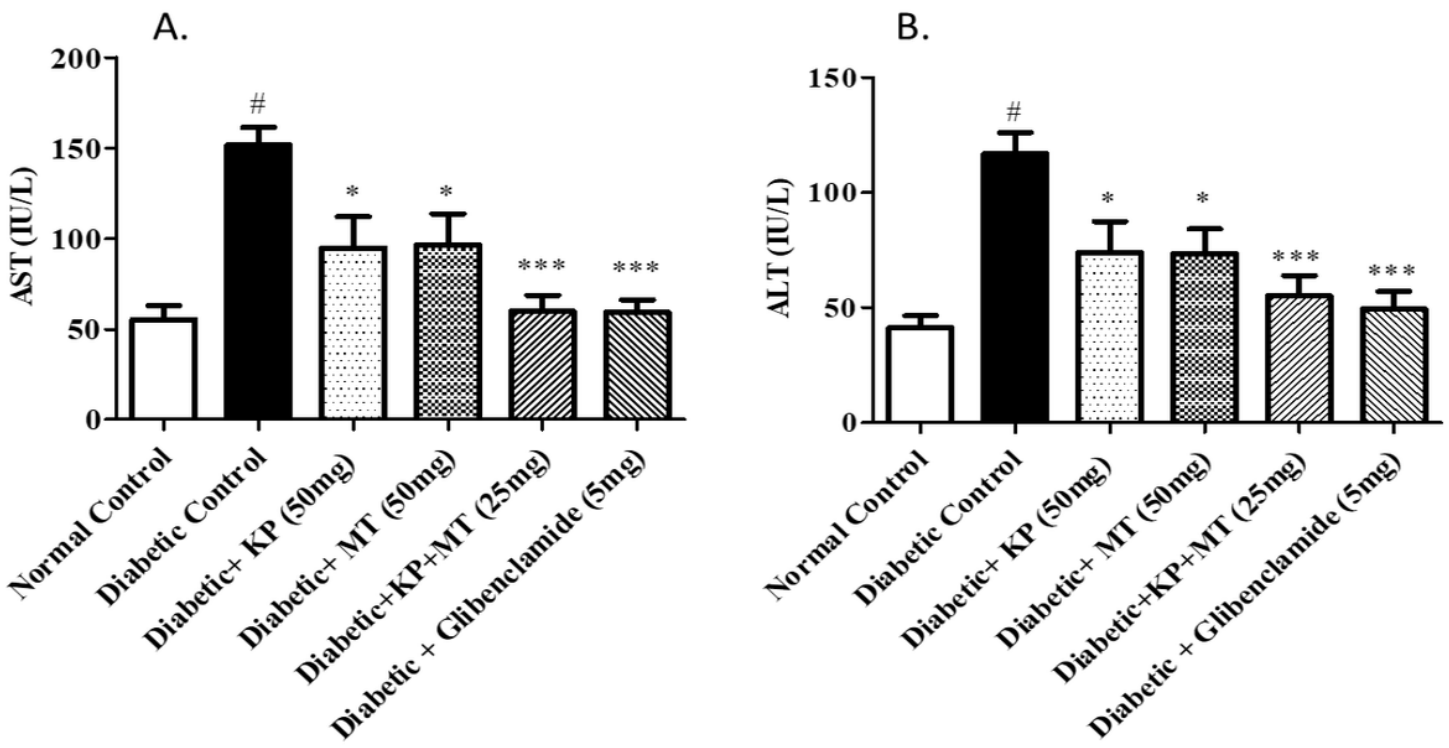

Figure 7

Effect of kaempferol and myricetin on liver enzymes A. AST, and B. ALT in streptozotocin-induced rats. All values are presented as mean ± SEM. Corelation amid the groups was done using Tukey's post comparison test by one- way ANOVA.

P value < 0.5, 0.01, 0.001 was expressed as *, **, *** as correlated to diabetic group

Signifies comparison to control group (P<0.001)

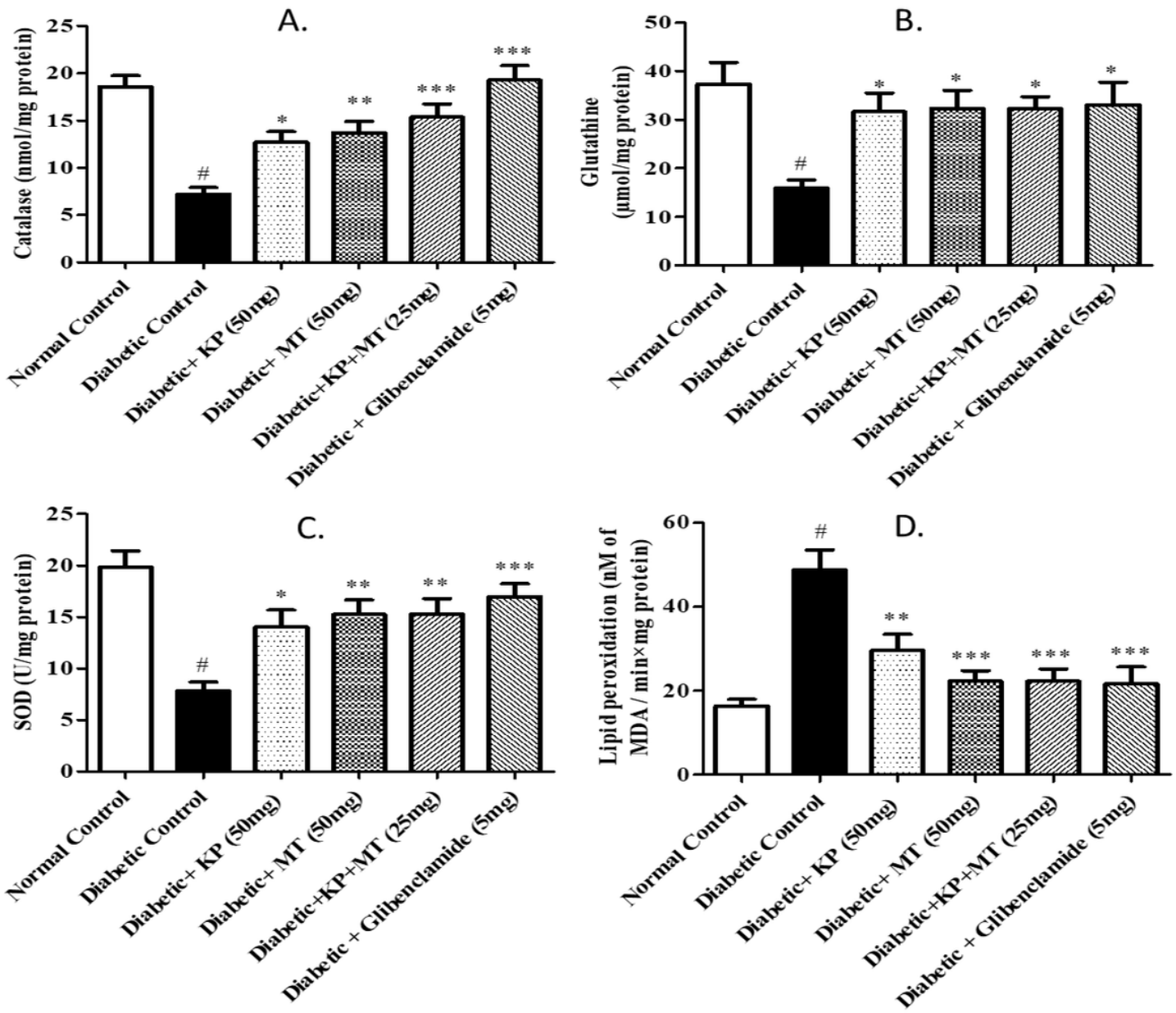

Figure 8

Effect of kaempferol and myricetin on antioxidant enzymes A. Catalase, B. GSH, C. SOD, and D. Lipid peroxidation (MDA) in streptozotocin-induced rats. All values are presented as mean ± SEM. Corelation amid the groups was done using Tukey's post comparison test by one- way ANOVA.

P value < 0.5, 0.01, 0.001 was expressed as *, **, *** when correlated to diabetic group

Signifies comparison to control group (P<0.001)

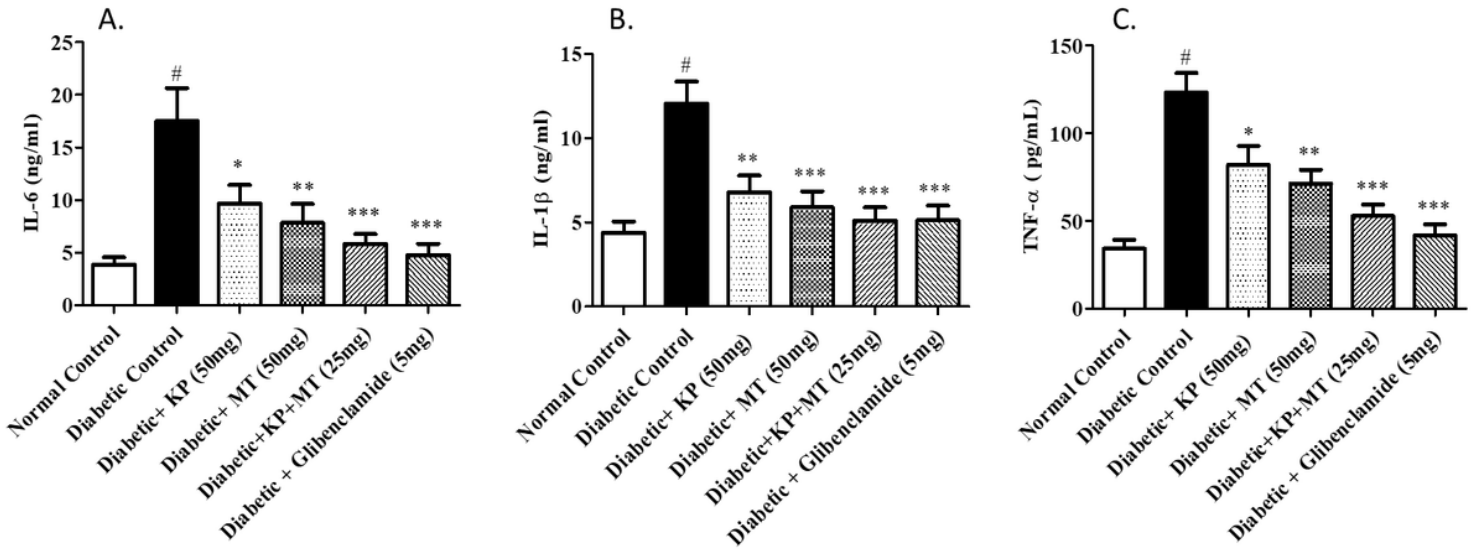

Figure 9

Effect of kaempferol and myricetin on inflammatory cytokines A. IL-6, B. IL-1β, and C. TNF-α in streptozotocin-induced rats. All values are presented as mean ± SEM. Corelation amid the groups was done using Tukey's post comparison test by one- way ANOVA.

P value < 0.5, 0.01, 0.001 was expressed as *, **, *** when correlated to diabetic group

signifies comparison to control group (P<0.001)

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