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Research Article
Oxidative Damage and Anti-Inflammatory Activity of Myricetin from Berries in Streptozotocin-Induced Diabetic Rats

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Abstract

Background and Objective: Myricetin has been investigated as an alternative treatment for diabetic rats induced by Streptozotocin (STZ). This study intends to find the efficacy of myricetin as an anti-inflammatory and oxidative effect on streptozotocin-induced diabetic rats. **Materials and Methods:** Single dose of STZ (55 mg kg^{-1} b.wt.) intraperitoneally was used to induce diabetes in rats followed by checking of various biochemical parameters and NF-κB protein levels in the kidney using hematoxylin-eosin (H and E) staining and immunohistochemistry. **Results:** The treatment, showed reduced glucose levels in fasting blood, lipid levels, Malondialdehyde (MDA) levels and inflammatory markers in diabetic rats kidneys as well as antioxidant enzyme levels. **Conclusion:** In diabetic rats, myricetin inhibits renal damage by reducing lipid parameters, inflammation and oxidative stress. This study provides evidence that myricetin can significantly reduce kidney damage and it provides evidence that this novel compound plays a significant role in kidney protection.

Key words: Blood glucose, serum, creatinine, urea, antidiabetic, malondialdehyde, myricetin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The prevalence of Diabetes Mellitus (DM), the metabolic disorder associated with impaired glucose tolerance, is growing globally[1]. Globally, approximately 366 million adults (20-79 years old) were diagnosed with DM in 2011 and this number is estimated to rise to 552 million by 2030, according to the International Diabetes Federation. Hyperglycemia and diabetic complications are characteristics of all forms of diabetes[2]. Infections as well as macrovascular and microvascular disorders such as cerebrovascular disorders, heart attacks, amputations, blindness and renal failure, can all be complications of diabetes[3]. Contemporary synthetic medicines are unable to control the course of diabetic complications for long periods and have profound effects on economic and social systems[4]. The control of DM and its complications requires novel treatments with minimal side effects[5].

Streptozotocin (STZ) and other b-cytotoxic drugs are commonly administered to experimental animals to induce diabetes[6]. Several types of pathogenicity have been observed following STZ induction of pancreatic b-cell necrosis. In type I diabetic models it is preferred since it does not lead to neurotoxicity (>55 mg kg^{-1} b.wt., of STZ), similar to alloxan. However, when administered at high doses, it can be lethal to the central nervous system[7].

In tea, berries, fruits, vegetables and medicinal herbs, myricetin (3, 5, 7, 3', 4', 5'-hexahydroxyflavone) is naturally occurring as a flavonoid[8,9]. Clinical and epidemiological studies have been conducted on myricetin and diabetes[10]. The association between myricetin and DM has been reported in several studies[11-13]. Researchers have proposed that myricetin is a primitive flavonoid found in the Chrysobalanaceae family that controls glycemia of diabetic patients in northern Brazil[14]. The plant extract containing myricetin has shown an antidiabetic and hypolipidemic potential as well as favourable effects against Non-alcoholic Steatohepatitis (NASH) in several recent studies[15]. Myricetin can also reduce adipocyte hypertrophy in the liver by increasing PPAR mRNA and cytochrome P450 protein levels. In some studies, myricetin has been shown to have anti-inflammatory properties, which suggests it has potential as a powerful anti-inflammatory agent[16].

On a streptozotocin-cadmium-induced diabetic model *in vivo*, myricetin enhanced both enzymatic and non-enzymatic antioxidant defence systems[17,18]. It may induce hyperglycemia and renoprotection by inhibiting intestinal (29%) and porcine (64%) α-glucosidase, however, it has no effect on α-amylase. The t-BHP-induced oxidative stress produced by the glutathione peroxidase and xanthine oxidase enzymes in the diabetic rat model is alleviated by myricetin[19]. Diabetes-related lipogenesis was stimulated in diabetic rat adipocytes by myricetin and insulin stimulation was enhanced by myricetin[20].

The hypoglycemic effect of myricetin is determined by *in vitro* animal studies. The present studies concentrate on DM and Myricetin's relationship. We discussed the underlying mechanisms, which may provide insight into how to treat DM.

MATERIALS AND METHODS

Study area: The study was carried out at Health Management Center, Central Hospital Affiliated to Shandong First Medical University between March, 2020-April, 2021.

Chemicals: The chemicals were acquired by Sigma Aldrich, St. Louis, MO, USA, two drugs that cause diabetes. Streptozotocin (CAS 18883-66-4) and myricetin (CAS 529-44-2) are two such agents. Abcam, Cambridge, UK, provided Superoxide Dismutase (SOD) (abs 65354), Glutathione-S-Transferase (GST) (abs 65326) and Catalase (CAT) (abs 83464). ELISA kits for the assay of inflammation by C-reactive protein (ab 108827), TNF-α (ab 46070), IL-1β (ab 100768) and IL-6 (ab 100772) were procured from Abcam, Cambridge, UK. Primary antibodies (TNF-α) and Goat Anti-Mouse IgG H and L (HRP) (Abcam, Cambridge, UK) used in this study were purchased from Abcam, Cambridge, UK. We used commercially available supplemental chemicals from high purity grades in our study.

Animals: We obtained 20 healthy Albino Wistar rats that weigh between 180-220 g. Polypropylene cages were used for the animals in the animal house with a temperature of 22°C, relative humidity of 50-60% and 12 hrs light/dark cycle. A standard pellet diet and *ad libitum* water were provided to the animals throughout the experiment and streptozotocin was used for the induction of diabetics in rats. The animal experiments were conducted following the approval from their Institutional Animal Ethical Committee.

Experimental design: We randomly selected eight animals for each group and divided them into 4 groups (group I through group IV). Before the experiment started, the animals had fasted overnight. Diabetes was induced in rats (groups II, III and IV) by a single intraperitoneal (i.p.) injection of freshly prepared STZ (55 mg kg^{-1} b.wt.) dissolved in 0.01 M citrate buffer, pH 4.5, except for group I animals, which received the

same buffer as the placebo. To test for hyperglycemia, tail vein punctures were used to measure blood glucose levels in control and diabetic rats. In the experiment, animals with fasting blood glucose levels of 200 mg dL^{-1} or higher were distinguished as diabetic after the 3rd day of the STZ injection. We began our treatment on day 4 following STZ injection but this was considered day 1 of treatment and lasted for 8 weeks. We divided 32 Albino Wistar rats into four groups at random. A standard pellet diet was provided to Normal Control (NC) rats in Group I. Fresh preparation of STZ (55 mg kg^{-1} b.wt.) was intraperitoneally injected into the rats in a citrate buffer of pH 4.5 as part of Group II Diabetes Control (DC). Myricetin (10 mg kg^{-1} b.wt.) was administered to the rats of Group III Diabetes Control+Myricetin (DC+Myr.). The diabetic rats were given a combination of DCC+Glibenclamide (5 mg kg^{-1} b.wt.) and the standard rats were given glibenclamide (80 mg kg^{-1} b.wt.).

Biochemical parameters and body weight measurement: To determine whether the overall weight of the animals has changed, we recorded the body weights of all four groups once a week, then analyzed the results. We collected blood samples and allowed them to clot for 30 min at room temperature, then centrifuged the serum at 3,500 rpm for 10 min at the end of the study. In this study, cholesterol, triglycerides and low-density lipoproteins were calculated and interpreted using Autoanalyzer. (Medi Lab 5, Shenzhen, China). Additionally, blood urea and creatinine were determined with reagent kits purchased from Sigma-Aldrich (St. Louis, Missouri, United States).

Defining the level of lipid peroxidation: MDA level assays were performed in duplicate on kidney homogenate of rats in the experimental group to determine whether or not lipid peroxidation was present.

Detection of antioxidant enzymes: Small portions of the kidney along with phosphate buffer saline were taken from all the rats. We homogenized all tissues and centrifuged them at 1100×g for 15 min at 4°C. Our laboratory measured the levels of several antioxidant enzymes, including SOD, GST, CAT and GSH, using kits available from the manufacturer, based on the manufacturer's instructions.

Measuring inflammation-related parameters: The manufacturer's protocol was followed for measuring CRP, TNF-α, IL-6 and IL-1β and the results were interpreted according to their interpretation.

An evaluation of the kidney's histopathology: An embedding block of paraffin-embedded kidney tissue samples was made from all of the fixed samples of kidney tissue in neutral formalin. Five-meter sections were thinly sectioned (5 m) and attached to slides. We stained kidney tissues with hematoxylin and eosin (H and E) to evaluate the architecture. Photographs of the slides were taken according to the images on a microscope slide. The light microscope used (Olympus, Tokyo, Japan) was used to conduct the histopathological examination. An independent histopathologist conducted the histological examination.

Expression of TNF-α: On the kidney, real-time PCR was used to measure the expression of the TNF-α gene from kidney tissue. Trizol (Invitrogen, Thermo Scientific, USA) was used to isolate total RNA from tissue samples according to the manufacturer's protocol. Total RNA purity and integrity were determined using Qubit 4 Fluorometer (Invitrogen, Thermo Scientific, USA) and agarose gel electrophoresis. The cDNA was made according to the manufacturer's instructions using the maxima first strand cDNA Synthesis Kit for RT-qPCR, with dsDNase (Thermo Scientific, USA). In brief, mixing 0.5 µg of total RNA with 1 µL of dsDNase, 1 µL of 10X dsDNase Buffer, total volume make up to 10 µL with nuclease-free water, gently mixed and centrifuged. The thermomixer was preheated to 37°C for 2 min to allow the reaction mixture to incubate. 4 µL reaction mix, 2 µL of maxima enzyme mix and 4 µL of nuclease-free water was added to a final volume of 20 µL. As per the manufacturer's instructions, reverse transcription reaction conditions were followed. An iTaq Universal SYBR Green Supermix kit (BIO-RAD laboratories, Inc, USA) was used for a real-time quantitative PCR using the CFX96 Touch Real-Time PCR Detection System (BIO-RAD Laboratories, USA). An actin gene was used as a housekeeping gene for normalization. In Table 1, you can find the primers for target genes and β-actin. The amplification was carried out in a final volume of 10 µL, which included 0.8 µL of cDNA template, 0.5 µL of forwarding primer, 0.5 µL of reverse primer, 5 µL of iTaq SYBR green master mix and 3.2 µL of nuclease-free water. The qPCR was carried out as follows: Initial denaturation for 30 sec at 94°C, followed by 40 annealing cycles at 94 and 56°C, with melting curve analysis performed

Table 1: Primers used for the target gene and actin in this study

Gene	Amplicon size	Primer sequence
β-actin	111 bp	Forward: 5-GAAGATCAAGATCATTGCTCCT-3′
		Reverse: 5-TACTCCTGCTTGCTGATCCA-3
TNF-α	120 bp	Forward: 5′-CTCTTCTGCCTGCTGCACTTTG-3′
		Reverse: 5′-ATGGGCTACAGGCTTGTCACTC-3′

after increasing the temperature from 65-95 °C. We calculated the relative expression levels of mRNA for samples based on the β-actin gene mRNA levels using the $2^{-\Delta\Delta}$Ct method[20].

Statistical analysis: We presented all data as Means±Standard deviations and we used one-way analysis of variance (ANOVA) to compare multiple groups. Statistical significance was determined by $p<0.05$ for a 2 tailed test.

RESULTS

Bodyweight and blood glucose levels are affected by myricetin: During the 8-week long experimental period, every rat group's body weight was measured every week. A significant reduction in body weight was observed in diabetic rats compared with control rats. When group III diabetic rats were continuously treated with myricetin, the weight loss levels were reduced. Myricetin-treated diabetic rats gained nearly 26.22% more body weight than the diabetes control rats (group II) (Fig. 1a-b). The data in Fig. 1a describe the change in the body weight of the animal model with the application of myricetin and Fig. 1b describe the change in the level of glucose after the application of the drug. The initial and the final body weight of different groups of experimental design (a). The rats were equally divided into 4 groups, each group (n = 8). Data are represented as Mean±Standard error of the Mean±SEM. Groups: Normal Control (NC), Disease Control (DC), i.e., STZ-treated group, treatment group (DC+Myr), Positive Control (PC) animal treated with STZ+Glibenclamide, *p<0.05 (significant difference of final b.wt., between DC vs. NC) #p<0.05 (significant difference of final b.wt., between DC vs. DC+Myr). The values of Fig. 1b indicate serum glucose in different groups of rats after 8 weeks of treatment. The rats were equally divided into 4 groups, each group (n = 8). Data are represented as the Mean±Standard error of the mean (SEM). Groups: Normal Control (NC), Disease Control (DC), i.e., STZ-treated group, treatment group (DC+Myr), Positive Control (PC) animal treated with STZ+Glibenclamide, *p<0.05 (significant difference of final b.wt., between DC vs. NC) #p<0.05 (significant difference of final b.wt., between DC vs. DC+Myr).

Myricetin's effect on lipid profile: Compared to those of the control group, the level of TG, TC and LDL-C were markedly higher in the diabetic rats induced by STZ. The TC, TG, LDL-C levels were significantly decreased in the myricetin (150 mg kg^{-1}) treated animal group compared to the STZ treated animals ($p<0.05$) (Fig. 2). The data in Fig. 2a describes the change in the cholesterol level in the mice model, Fig. 2b describes the change in the triglyceride level in the mice model whereas Fig. 2c describes the change in the LDL cholesterol levels after the application of myricetin. The rats were equally divided into 4 groups, each group (n = 8). Data are presented as the mean standard error of the Mean±SEM.

Groups: Normal Control (NC), Disease Control (DC), i.e., STZ-treated group, treatment group (DC+Myr), Positive Control (PC) animal treated with STZ+Glibenclamide, *p<0.05 (significant difference of final b.wt., between DC vs. NC) #p<0.05 (significant difference of final b.wt., between DC vs. DC+Myr).

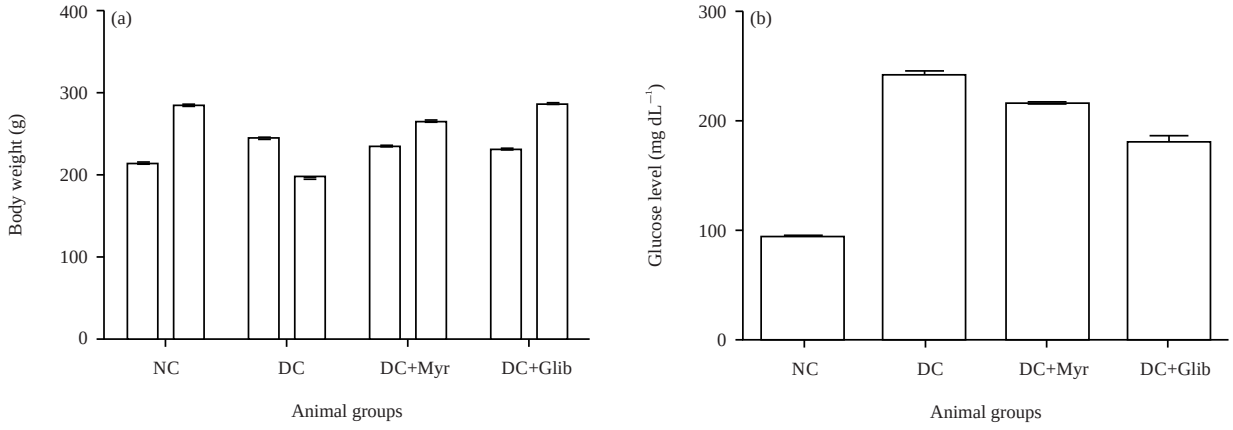

Fig. 1(a-b): Body weight and glucose level of myricetin treated and untreated rats, (a) Change in the body weight of the animal model and (b) Change in the glucose level

NC: Normal control, DC: Disease control, i.e., STZ-treated group, treatment group (DC+Myr), PC: Positive control and animal treated with STZ+Glibenclamide (DC+Glib)

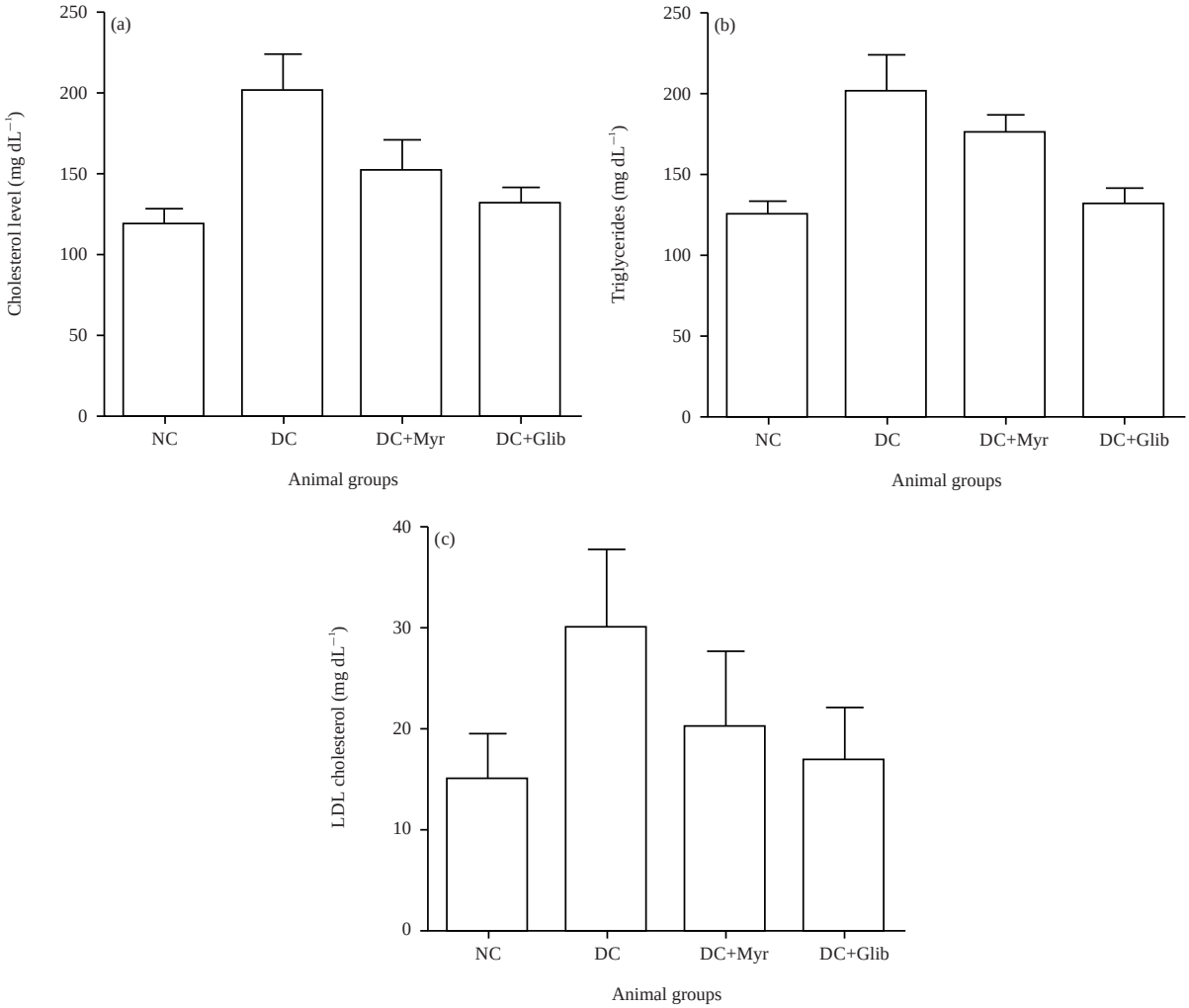

Fig. 2(a-c): Lipid profile in different groups of rats after 8 weeks of continuous treatment, (a) Change in the cholesterol level in the mice model, (b) Change in the triglyceride level in the mice model and (c) Change in the LDL cholesterol level in the mice model

NC: Normal control, DC: Disease control, i.e., STZ-treated group, treatment group (DC+Myr), PC: Positive control and animal treated with STZ+Glibenclamide (DC+Glib)

Effects of myricetin on blood urea and serum creatinine: A significant difference between the STZ group and the control group was observed in serum creatinine and blood urea following the induction of hyperglycemia with STZ. Compared to STZ-treated animals, myricetin treatment significantly reduced serum creatinine and blood urea, two critical biomarkers associated with kidney dysfunction. In rats with diabetes induced by STZ, myricetin protects rental function (Fig. 3a-b). The data in Fig. 3a describes the change in the level of creatinine whereas Fig. 3b describes the change in urea level within the mice model with the application of myricetin.

Myricetin's effect on oxidative stress: Myricetin was found to protect rat redox status by reducing MDA levels as well as antioxidant enzyme levels (catalase, enzymes of oxidation, GST and glutathione) (Fig. 4). STZ-induced diabetic rats had significantly lower CAT, SOD, GST and GSH levels than control rats. As a result of reversing the effects of STZ induced oxidative stress, the antioxidant levels of diabetic animals treated with myricetin were increased (Fig. 5). When compared to the control rats, diabetic rats with STZ-induced diabetes had significantly higher kidney MDA levels. The presence of MDA has been significantly decreased in diabetic rats treated with myricetin (Fig. 4). The data in Fig. 5 describes the antioxidant property of myricetin in which Fig. 5a changes in catalase, Fig. 5b change in SOD, Fig. 5c change in GSH and Fig. 5d change in the level of GST.

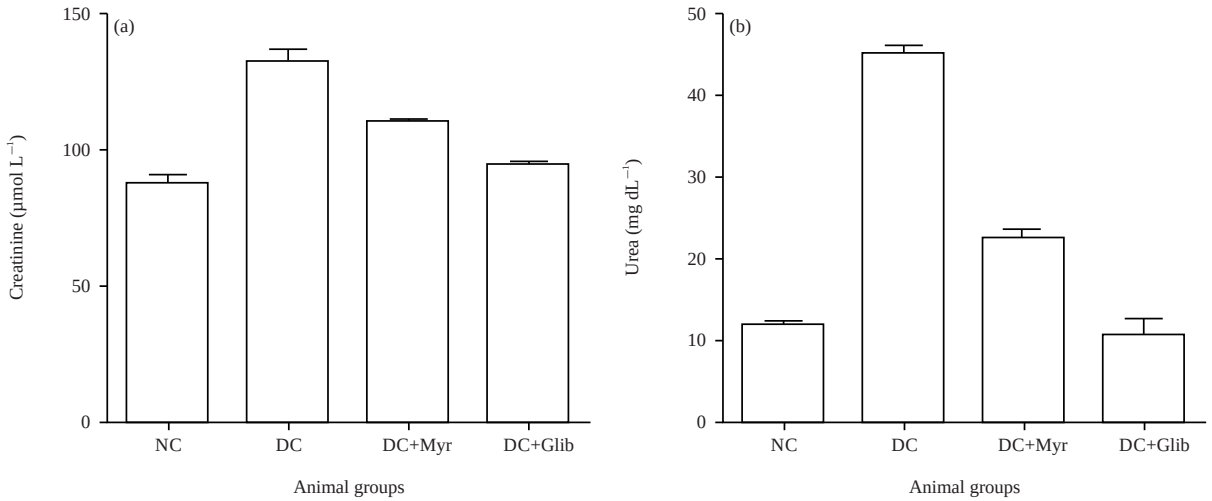

Fig. 3(a-b): Kidney function test profile (creatinine and urea) in different groups of rats after 8 weeks of treatment, (a) Change in the creatinine level in the model mice and (b) Change in the level of urea in the model mice

NC: Normal control, DC: Disease control, i.e., STZ-treated group, treatment group (DC+Myr), PC: Positive control and animal treated with STZ+Glibenclamide (DC+Glib)

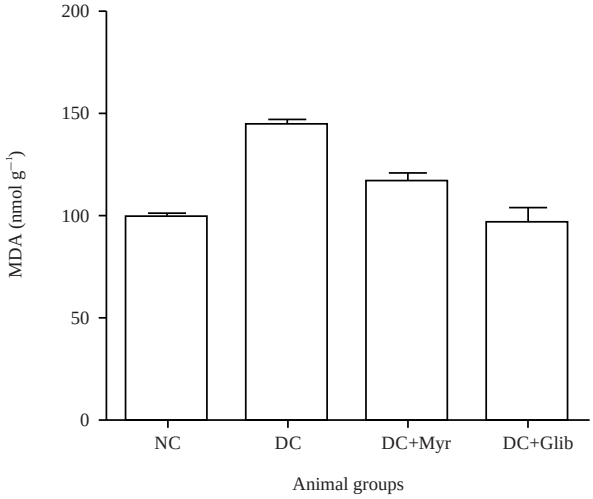

Fig. 4: Lipid peroxidation (MDA) level in different groups of rats after 8 weeks of treatment

Rats were equally divided into 4 groups, each group (sample size = 8), Data are shown as Mean±Standard error of the Mean±SEM, NC: Normal control, DC: Disease control, i.e., STZ-treated group, treatment group (DC+Myr), PC: Positive control and animal treated with STZ+Glibenclamide (DC+Glib)

Myricetin decreases TNF-α, CRP, IL-6 and IL-1b levels: When compared with normal rats, the diabetic rats showed noticeable inflammatory responses in the kidneys, CRP, IL-6, IL-1β and TNF-α, levels were significantly higher in diabetic rats ($p<0.05$). As compared to diabetic rats treated only with STZ, rats treated with myricetin had significantly lower levels of inflammatory markers in their kidneys (Fig. 6). The data in Fig. 6a describes the change in TNF-α, Fig. 6b change in IL-6, Fig. 6c IL-1β and Fig. 6d change in CRP level on the application of myricetin.

Physiological effects of myricetin on kidneys: Staining of hematoxylin-eosin (H and E) revealed the kidney's architecture. The kidney tissue of rats in the control group exhibited normal glomeruli and renal tubules. Diabetic rats showed glomerular basement membrane thickening, infiltration of lymphocytes and congestion in comparison to normal control rats. A study that administered myricetin to rats for 8 weeks showed a marked decrease in deformity in both the glomerular and tubular architecture as well as mild inflammation and congestion. Treatment with myricetin significantly decreased the damages to the kidneys, further indicating that myricetin contributes significantly to protection against kidney damage. When compared to the diabetic control group, myricetin significantly reduced collagen deposition (Fig. 7). The data in Fig. 7a shows the typical kidney architecture of controls, Fig. 7b STZ only treated kidney tissues had infiltrated lymphocytes and thickened glomerular basement membranes Fig. 7c the presence of myricetin markedly compromised glomerular and tubular architectures and mild congestion was observed. The data in Fig. 7d Positive control (diabetic rats treated with glibenclamide) showing normal kidney architecture Fig. 7e myricetin-treated animals.

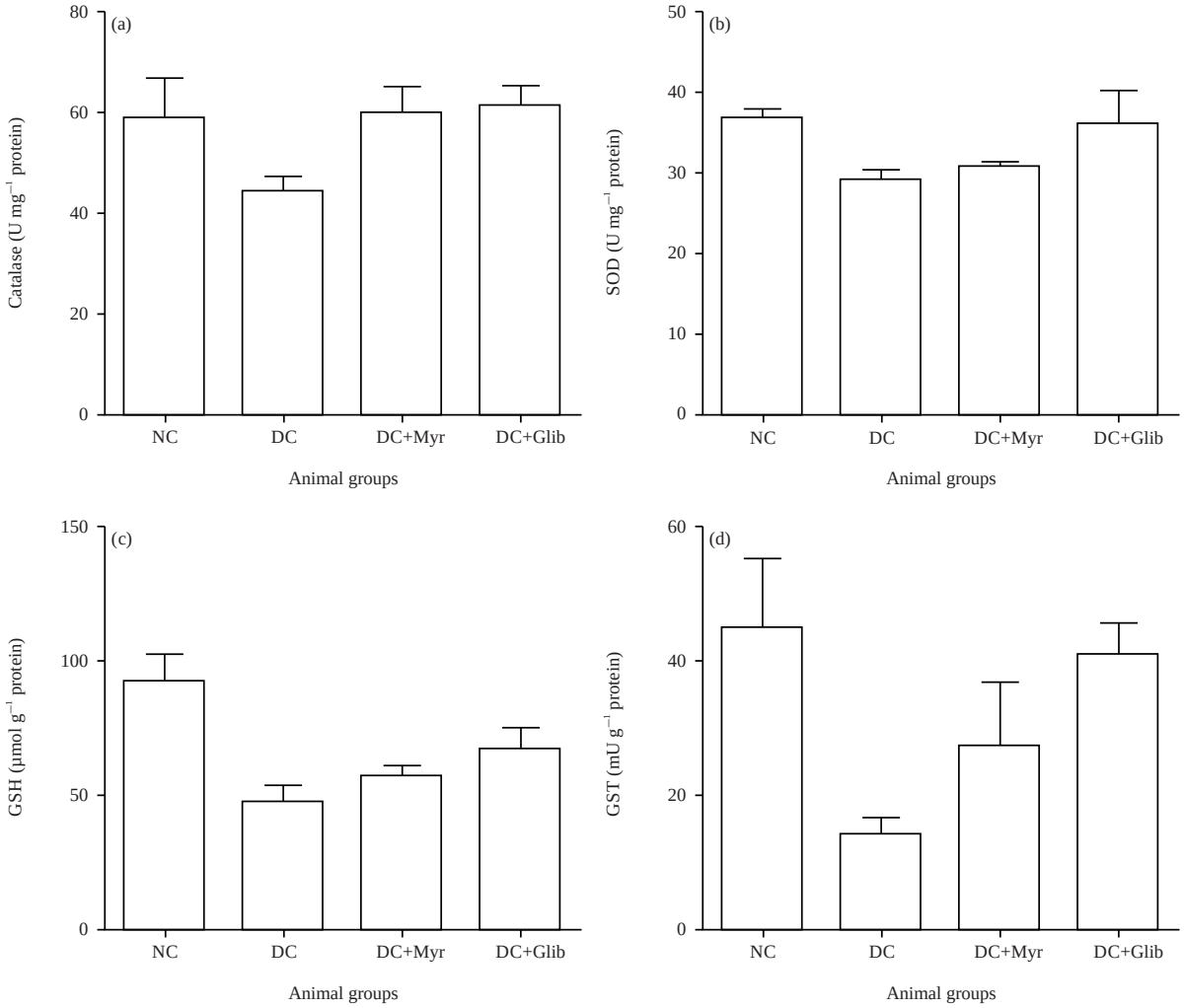

Fig. 5(a-d): Antioxidant enzyme, (a) Change in the catalase, (b) Change in SOD, (c) Change in GSH, (5) Change in GST level in different groups of rats after 8 weeks of treatment

Rats were equally divided into 4 groups, each group (sample size = 8). Data are shown as the mean standard error of the mean (SEM), NC: Normal control, DC: Disease control, i.e., STZ-treated group, treatment group (DC+Myr), PC: Positive control and animal treated with STZ+Glibenclamide (DC+Glib)

Expression of TNF-α by myricetin: When compared to the control group, the diabetic control group showed an intense expression of TNF-α protein ($p < 0.01$). Diabetes with myricetin group showed lower expression in comparison to diabetic control group than diabetic with myricetin group ($p < 0.05$). The data in Fig. 8 describes diabetic rats were treated with myricetin, TNF-α expression reduced significantly as well as in diabetic rats.

DISCUSSION

In this study, we evaluated oxidative stress, inflammation, histopathological and biochemical alterations of rats with STZ-induced renal damage in the present study. Myricetin inhibits renal damage by modulating a variety of biological activities and pathogenesis including renal damage[21]. In addition, myricetin inhibits inflammation and promotes insulin secretion in beta cells via GLP-1 modulation and up regulation of Rab27a/Slp4-a in pancreatic beta cells[22,23].

It also enhances GLUT4 membrane presentation and increases glycogen synthase 1 in skeletal muscle, enabling glucose disposal. In addition to reducing oxidative stress and inflammation, myricetin promotes nephroprotective effects[24]. In this study, the drug myricetin has also been found to negatively regulate blood glucose, creatinine and blood urea nitrogen levels. As well as controlling pro-inflammatory cytokines, Nuclear Factor kappa B (NF-κB) activation, p38MAPK activity in the renal cortex and NF-κB activity, it also regulates

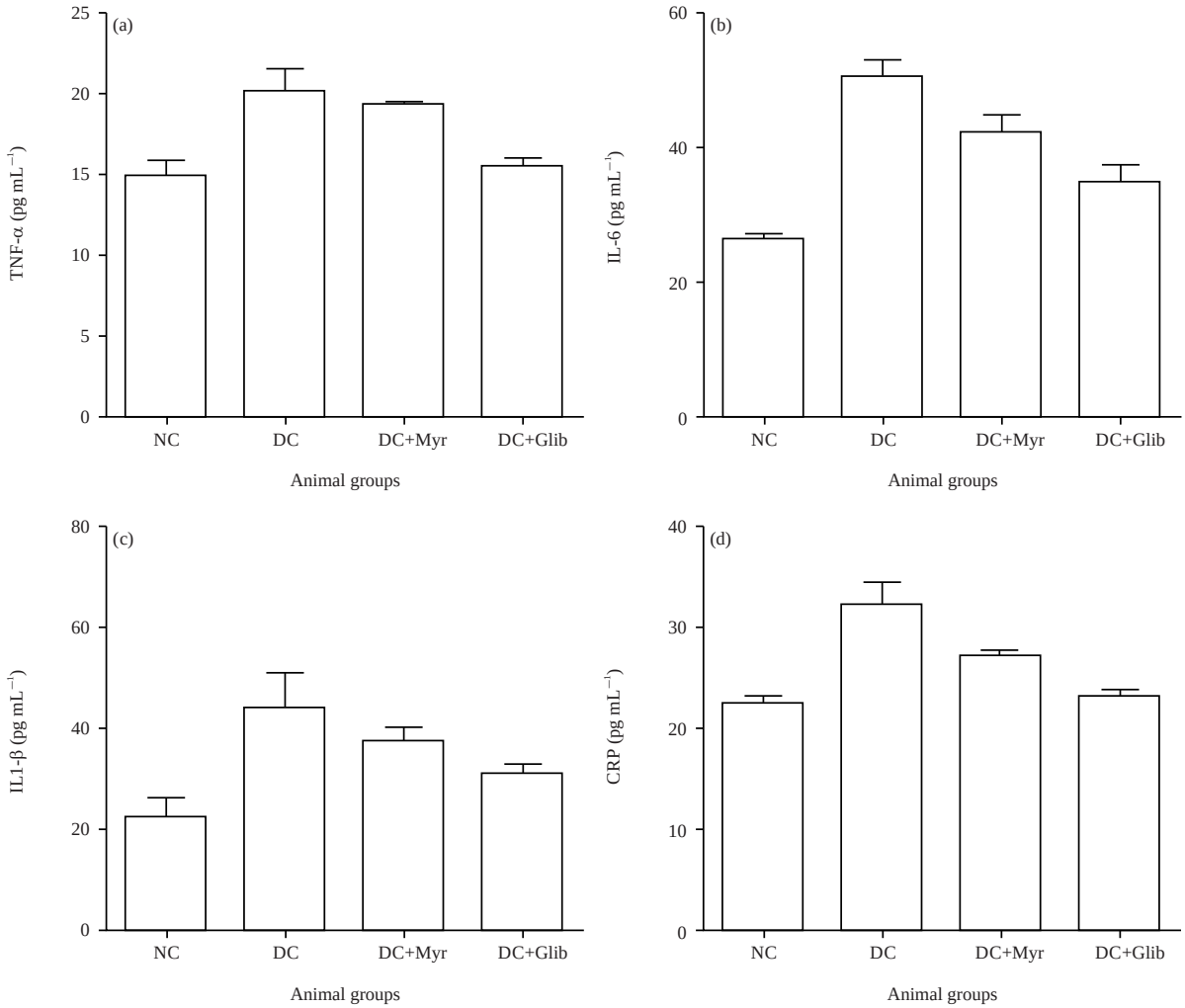

Fig. 6(a-d): Inflammatory markers level in different groups of rats after 8 weeks of treatment, (a) Variation of TNF-α, (b) Variation of IL-6, (c) Variation of IL1 beta and (d) Variation of CRP

Rats were equally divided into 4 groups, each group (sample size = 8). Data are summarized as the mean standard error of the mean (SEM), NS: Normal control, DC: Disease control, i.e., STZ-treated group, treatment group (DC+Myr), PC: Positive control, animal treated with STZ+glibenclamide (DC+Glib)

inflammation and the response to oxidative stress[25]. When blood glucose levels are high after fasting, it is called diabetes mellitus. Diabetes mellitus establishes risk factors for coronary heart disease[26]. The fasting blood glucose concentration in the STZ group animals was significantly higher as compared to the control group ($p<0.05$). In comparison to the STZ group, the fasting blood sugar levels in the STZ plus myricetin group were significantly decreased ($p<0.05$) (Fig. 1b). It is also associated with the regulation of cholesterol metabolism, including its synthesis and absorption, that a proper insulin level is required. STZ-induced diabetic rats had significantly higher levels of TGs, TCs and LDL-C than the control group in the present study. The lipid profile of animals treated with myricetin (150 mg kg^{-1}) was significantly reduced in comparison with animals treated with STZ (Fig. 2). A significant reduction in serum creatinine and blood urea was also observed when myricetin was administered in comparison to STZ. The results of this study indicate the protection of rental function by myricetin in STZ-induced diabetes (Fig. 3). Other studies have demonstrated a dose-dependent reduction in blood glucose levels, creatinine levels and blood urea nitrogen levels with myricetin. Its anti-inflammatory and antioxidant properties have also been proven. Activated Reactive Oxygen Species (ROS) are the key cause of kidney disease because of an imbalance between oxidation and antioxidants. There is evidence that hyperglycemia may impair the ability of renal epithelial cells and glomerular mesangial cells to produce ROS, damaging

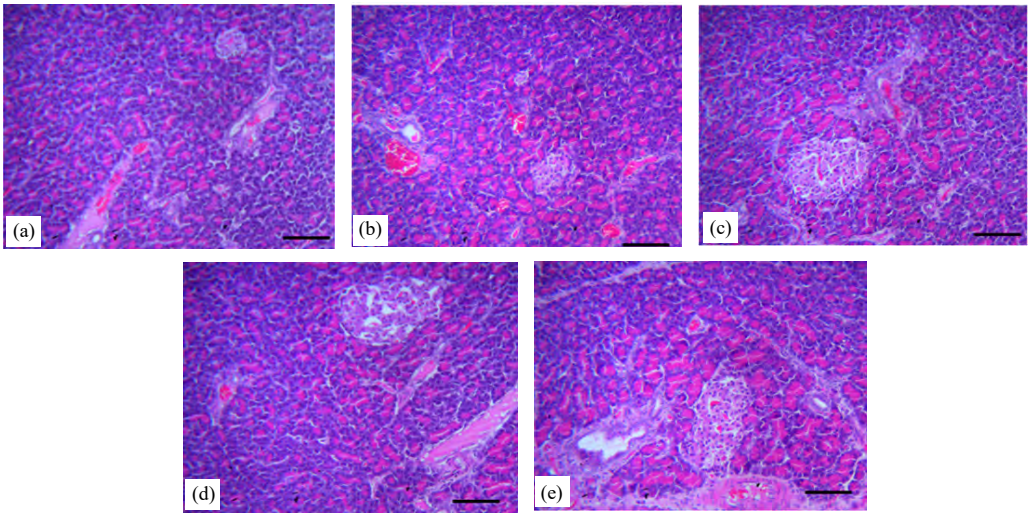

Fig. 7(a-e): Histopathological changes in kidney tissues in response to myricetin, (a) Shows the typical kidney architecture of controls, (b) STZ only treated kidney tissues had infiltrated lymphocytes and thickened glomerular basement membranes, (c) Presence of myricetin markedly compromised glomerular and tubular architectures and mild congestion was observed, (d) Positive control (diabetic rats treated with glibenclamide) showing normal kidney architecture and (e) myricetin-treated animals
Scale bar = 100 μm

tissue proteins, increasing lipid peroxide production and aggravating renal oxidative damage[27]. In addition, hyperglycemia has been shown to result in oxidative injury via raising oxidative stress, resulting in excessive ROS production. Furthermore, the rate of oxygen consumption in kidney tissues makes them more vulnerable to ROS related to oxidative damage[28]. As a result of excess ROS, MDA content could also be enhanced, an indicator of lipid peroxidation[29]. Using myricetin, we observed that the elevated levels of antioxidants in STZ-induced diabetic mice were significantly improved. A significant increase in urinary MDA levels was also found in STZ-induced diabetic rats. Diabetes-induced kidney injury in mice treated with myricetin was significantly reduced (Fig. 4). Similarly, an animal study conducted on diabetic animals showed reduced lipid peroxidation and nitrosative stress when diabetic mice were administered gentamicin in an enriched solution of myricetin. The presence of myricetin can ameliorate the oxidative stress caused by gentamicin in the renal cortex in addition to an increase in GSH and SOD activity[30]. Ginger also has been described as a reno-protective agent with a function in regulating lipid peroxidation and maintaining histopathological changes[31]. Myricetin was shown to lower lipid peroxidation, increase the antioxidant capacity and reduce renal nephropathy in this study (Fig. 4-6). Diabetes mellitus is caused by over expression of inflammatory cytokines. Diabetes is a disease in which inflammation is a key factor. Pro-inflammatory cytokines make diabetic nephropathy more likely to occur, resulting in changes in glomerular filtration rate and endothelial cell permeability and the formation of ROS and free radicals. Through inhibition of inflammatory processes, natural products or active compounds of medicinal plants can inhibit pathogenesis[32,33]. A significant difference was found between diabetic and healthy rats in their inflammatory markers, in the present study. When myricetin was administered to diabetic rats in comparison to STZ-treated rats, there was a remarkable reduction in inflammatory markers in the kidney (Fig. 5). These studies suggest that myricetin treatment improved diabetic rats' kidney inflammation. Additionally, in a previous study, myricetin was found to improve renal tissue condition through changes in NF-κB activity and p38MAPK activity and to control the inflammatory response and oxidative stress[34].

Histopathological analyses of the kidney sections from the STZ treated group of rats demonstrated the presence of large pathological changes, such as oedema, inflammation, fibrosis and lymphocytic infiltration, which were not evident in the healthy control group. The present findings are in line with previous studies indicating that diabetic animals' kidneys are severely damaged, along with glomerular sclerosis. In rats administered myricetin, glomerular and tubular architecture abnormalities showed a marked reduction and there were mild degenerative changes and minimal fibrosis in the renal glomeruli (Fig. 7). By treating the kidney damage with myricetin, the damage to the kidneys was significantly

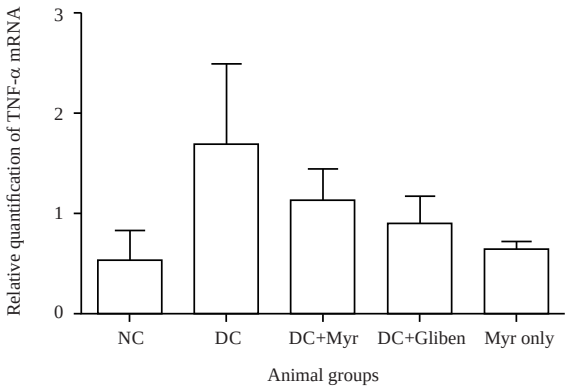

Fig. 8: TNF-α expression is shown graphically

Expression pattern was high in the diabetic control as compared to the normal control group (**p<0.01). A comparison of the diabetic group treated with myricetin and the diabetic control group shows lower expression (*p<0.05). Compared to the control group, the TNF-α expression pattern in the myricetin-treated animals and positive controls were statistically insignificant (p>0.05). NC: Normal control, DC: Disease control, i.e., STZ-treated group, treatment group (DC+Myr), PC: Positive control, animal treated with STZ+Glibenclamide (DC+Glib)

reduced. This has been shown to confirm the protective effects of myricetin. Compared to the normal control group, the diabetic control group showed high levels of TNF-α protein expression. The expression of TNF-α protein was significantly lower in diabetic groups treated by myricetin (Fig. 8). The drug significantly reduced the expression levels of TNF-α protein in diabetic rats and probably ameliorates renal damage by down regulating the TNF-α protein. We studied the effects of Ursolic acid, a commonly found triterpenoid compound, on some parameters related to renal disease[35]. Acute Ursolic acid administration reduced renal structural irregularities and raised levels of TNF-α, MCP-1 and IL-1β expression[36].

In conclusion, our findings suggest that myricetin prevents weight loss in diabetic rats and decreases blood glucose levels. It also shows retention protective effects in diabetic rats by regulating urea and creatinine levels, inhibiting oxidative stress, hyperglycemic and inflammatory markers such as TNF-α, IL-6, IL-1β and CRP. In addition, myricetin reduces the expression of the TNF-α protein in streptozotocin-induced diabetes and thereby ameliorates renal fibrosis and pathological changes. Clinical trials on its hypoglycemic effects and specific dosage are needed to verify its pharmacokinetics and bioavailability.

CONCLUSION

The study showed that myricetin was able to significantly prevent the damage of the kidney by reducing the levels of oxidative stresses, lipid parameters as well as inflammation. Thus myricetin can be used as an alternate therapeutic in treating such diabetic conditions.

SIGNIFICANCE STATEMENT

This study discovered myricetin can be beneficial for the treatment of diabetic patients preventing renal failure. This study would provide researchers and people of the medicinal sector with an alternative therapeutic strategy.

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