THERAPEUTIC POTENTIAL OF MYRICETIN IN STREPOZOTOCIN INDUCED DIABETES IN RATS.

Mahesh Bhanudas Narkhede[1]*, Vaibhav Suresh Adhao[2]

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Abstract

Background: Oxidative stress has been considered as contributory aspect for major complications of diabetes mellitus consisting of diabetes. The aim of this study was to examine the therapeutic effect of Myricetin in streptozotocin (STZ) induced diabetes through measuring biochemical parameters, oxidative indicators and histopathological examination of renal tissues**.**

Results: Administration of dose of STZ (55 mg/kg of body weight) intraperitoneal ended in diabetes in rats as indicated by increase in serum glucose level. Besides, STZ treatment led to depletion of antioxidant enzymes together with superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT). Administration of Myricetin at the dose of 1, 2 and 5 mg/kg extensively decreased biochemical parameters. Myricetin was observed to improved antioxidant enzymes ranges and offered safety in opposition to lipid peroxidation (LPO).STZ administered rats shows elevated level of TNF-α and IL-6 and Myricetin treated rats inhibited the elevated level of cytokine.

Conclusion: This study concluded that Myricetin may additionally modulate oxidative stress and protected renal tissues from STZ injury. Improvement in renal histopathological architecture further confirmed *in vivo* antioxidant activity of Myricetin in STZ induced diabetes.

Key words: Diabetes; Myricetin; Antioxidant activity; Oxidative stress

[1*,2]Department of Pharmacology, Dr Rajendra Gode College of Pharmacy, Malkapur Buldana Road Malkapur Dist Buldana 443101

*Corresponding Author: Mahesh Bhanudas Narkhede

*Department of Pharmacology, Dr Rajendra Gode College of Pharmacy, Malkapur Buldana Road Malkapur Dist Buldana 443101. Email: maheshnark@gmail.com, 9860297535, https://orcid.org/0000-0001-5899-6881

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Background

Diabetes also termed as high blood glucose disorder is a first-rate difficulty of lengthy status diabetes mellitus. Nearly 5 % patients of diabetes expand because of continual hyperglycemia[1]. Persistent hyperglycemia ends in progressive structural changes inside the renal tissues such as extracellular accumulation in the mesangium, glomerular basement membrane and tubule interstitial tissue[2]. Persistently high glucose level induces oxidative stress leading to production of reactive oxygen species (ROS) which can be responsible for renal cellular injury. Current treatment modalities for diabetes primarily focus on glycaemic and lipid control or lifestyle changes[3]. These therapeutic approaches are insufficient to control oxidative stress and subsequent progression of diabetes. Hence there is an urgent need to explore underlying mechanism and novel treatment for diabetes[4].

Myricetin is a naturally occurring flavonol with hydroxyl substitutions at the 3, 5, 7, 3', 4' and 5' positions. Its occurrence in nature is widespread among plants including tea, berries, fruits, vegetables and medicinal herbs[5]. Myricetin's occurrence in berries, vegetables and fruits is mostly in the form of glycosides rather than free aglycones and its content in berries increases considerably as the berries ripen. Myricetin is commonly consumed in our diet in vegetables, fruits and beverages such as tea and wine. Some of the consumed myricetin is absorbed by the gastrointestinal tract, whereas the remainder is metabolized by the gastrointestinal microflora[6]. The liver is largely responsible for the metabolism of the absorbed myricetin, with the intestinal wall and kidney as the secondary sites. The major metabolite from its metabolism has been identified as 3,5-dihydroxyphenylacetic acid, which is excreted in the urine[7]. Through the years, a number of studies have been done to investigate its varied therapeutic potential, which includes its use as a potent antioxidant, as an anticarcinogenic agent and in the prevention of platelet aggregation[8].The aim of this study was to investigate the anti diabetic effect of Myricetins in STZ induced diabetes in rats.

Methods

Animals

Thirty-six male Albino Wistar rats weighing 180-200 gm had been used for this experimental examine. Rats were procured from animal residence facility of Dr Rajendra Gode college of Pharmacy, Malkapur. Animals were placed in nicely-ventilated polypropylene cages and maintained under ambient temperature of 25 ± 2 °C, 12 hours light/dark cycle in the departmental animal house. The animals had been fed with trendy pelletized feed (Amrut Rat Feed, Pune) and water ad libitum. Experimental protocol was pre-accepted by institutional animal ethical committee (IAEC; 1336/ac/10/cpcsea/03).

Chemicals and drugs

STZ, Myricetin, metformin and carboxymethyl cellulose were obtained from Sigma Aldrich (Mumbai, India). Glucose kit, from ERBA (Mumbai, India). Tumor Necrosis factor- alpha (TNF-α) and Interlukine-6 (IL-6) were purchased from Loba Chemicals Pvt Ltd (Mumbai, India). All other chemicals used in the study were of analytical grade.

Induction of diabetes

Diabetes was induced in rats by a single dose of STZ (55 mg/kg) intraperitoneal route except normal group. STZ was prepared in cold phosphate buffer solution (pH- 7.4) in dark condition. After 72 hours of STZ administration, diabetes was induced in animals[9].

Experimental design

Rats were randomly divided into six groups (n=6).
Group 1: Normal - received 0.5% CMC
Group 2: Diabetic group - injected with STZ (55 mg/kg)
Group3: DIABETES + Standard drug (Metformin) - injected with STZ (55 mg/kg) + metformin (70 mg/kg)
Group 4 to 6: Injected with STZ (55 mg/kg) and 1, 3, 5 mg/kg of Myricetin respectively.

Metformin and Myricetin suspension was prepared in 0.5 % CMC and administered by oral route for 28 day. On 15th day animals were anesthetized by urethane (1.4 mg/kg) and blood samples were collected. For separation of serum blood was centrifuged at 5000 rpm for 1 min at 4 °C and serum was separated and used for further biochemical estimation. Finally animals were scarified and both kidneys were isolated. A 1% tissue homogenate of kidney was prepared using ice-cold 50 mM phosphate buffer saline (pH 7.4). The homogenate was centrifuged at 1000 rpm for 1 min at 4 °C and supernatant was separated and used for further estimation[10].

Physiological parameters

Body weight of each animal was recorded weekly by using electronic weighing balance. At the end of the experiment weight of both kidney and liver was taken.

Determination of blood glucose level

Serum glucose level was measured by the glucose oxidase-peroxidase(GOD-POD) method using the commercially available kit (Erba kit)[9].

Estimation of SGPT and SGOT

The level of SGPT and SGOT was determined according the manufactures protocol[9]

Cytokine parameters

Proinflammatory cytokines like TNF-α and IL-6 was measured in liver homogenate by using respective kits according to manufactures protocol and final concentration was determined by using standard curve[11].

Estimation of MDA content

Acetic acid (20%, pH 3.5) 1.5 ml, thiobarbituric acid (0.8%), sodium lauryl sulfate (8.1%) 0.2 ml were added to 0.1 ml of supernatant obtained above. Thereafter the organic layer was introvert and absorbance measured at 532 nm[12]

Statistical analysis

Data were presented as mean ± S.E.M. of each group. The result values were statistically analysed by using one-way ANOVA and two-way ANOVA followed by Bonferroni's test using graph pad prism version 7.0. A difference was regarded as significant when $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

Effect of STZ and Myricetin on change in body and organ weight

Body weight of the animals significantly decreased in STZ treated group as compared to normal group. Treatment with Myricetin increased the body weight as compared to STZ injected group ($p<0.001$) shown in figure 1. Whereas, the metformin treated group showed increase in the body weight as compared to the STZ group ($p < 0.001$). Meanwhile, STZ injected rats showed decrease in liver weigth (table 1 and increase in liver weight as compared to normal rats. But the entire tretament group showed reverse effect of STZ.

Table: 1 Effect of Myricetin on change in body and organ weight

Group	O day	7 th day	14 th day	21st day	28th day
Normal	180.45 ± 4.35	185.61 ± 5.26	187.21 ± 5.27	191.47 ± 4.32	202.12 ± 2.34
Diabetic	192.74 ± 7.15###	185.34 ± 6.45###	180.55± 7.84###	171.61 ± 5.42###	155.25±10.62###
Standard	207.49 ± 4.26	209.19 ± 5.46	212.49 ± 6.52**	215.74 ± 5.35***	217.65 ± 6.17***
Myricetin 1	201.31 ± 6.48	201.49 ± 9.65	195.61 ± 5.34	203.79 ± 6.24	204.26 ± 4.62
Myricetin 3	205.76 ± 7.19	205.64 ± 4.18	206.42 ± 6.25	207.84 ± 5.26	208.84 ± 6.35**
Myricetin 5	201.65 ± 6.75	199.67 ± 5.12	203.22 ± 4.62	207.26 ± 4.15**	209.65± 5.48***

Data were presented as mean ± S.E.M. (n-6). The result values were statistically analysed by using one-way ANOVA and two-way ANOVA followed by Bonferroni's test for multiple comparisn.

Effect of Myricetin on Blood glucose level

Blood glucose level was monitored weeklyand it was found that diabetic mice exhibited significantly increased plasma glucose levels ($p < 0.01$) as compared with the normal group at 1st, 2nd, 3rd and 4th week. Treatment with Nothofagin alone prevented such rise in blood glucose level significantly at Myricetin (3 and 5 mg/kg) ($p < 0.01$) in dose dependent manner at 1st, 2nd, 3rd and 4th week. Treatment with the Nothofagin 1 mg/kg also significantly ($p < 0.05$) at 1st, 2nd, 3rd and 4th week prevented such rise in blood glucose level. myrcietin treated with 2 mg/kg dose good effect on blood glucose level at 4th week shown in table 1.

Table 1: Effect of Myricetin on Blood glucose level

Group	Blood glucose level mg/dl				
	O day	7 th day	14 th day	21st day	28th day
Normal	95 ± 4.54	96 ± 6.52	96 ± 4.25	97 ± 5.18	97 ± 5.61
Diabetic	255 ± 8.41###	263 ± 7.55###	279 ± 5.96###	281 ± 9.65###	283 ± 11.25###
Standard	256 ± 8.67	196 ± 8.25	180 ± 6.35***	143 ± 6.24***	111 ± 7.51***
Myricetin 1	254 ± 9.65	251 ± 7.15	224 ± 4.19	197 ± 7.18	183 ± 8.44**
Myricetin 3	253 ± 6.35	235 ± 6.35	206 ± 5.76*	189 ± 6.97**	153 ± 7.15***
Myricetin 5	257 ± 8.94	205 ± 7.85	192 ± 5.68**	181 ± 7.19***	123 ± 8.65***

Values are expressed as means ±SEM, n = 06. Statistical significance was determined by one-way ANOVA followed by the Bonferroni's post hoc test: Compared with Normal ##P < 0.01, Compared with STZ* P < 0.05, **P <0.01.

Effect of Myricetin on serum biochemical parameter

The biochemical parameters like SGPT, SGOT were significantly (P<0.001) altered in STZ-induced diabetic rats compared to normal control rats. In diabetic rats, administration of both doses of Myricetin and Metformin significantly (P<0.001) reduced SGOT and SGPT levels compared to diabetic control rats. The Myricetin 5 mg/kg treatment showed significantly (P<0.001) higher reduction of SGOT and SGPT levels compared to Myricetin 1 mg/kg dose shown in figure 1.

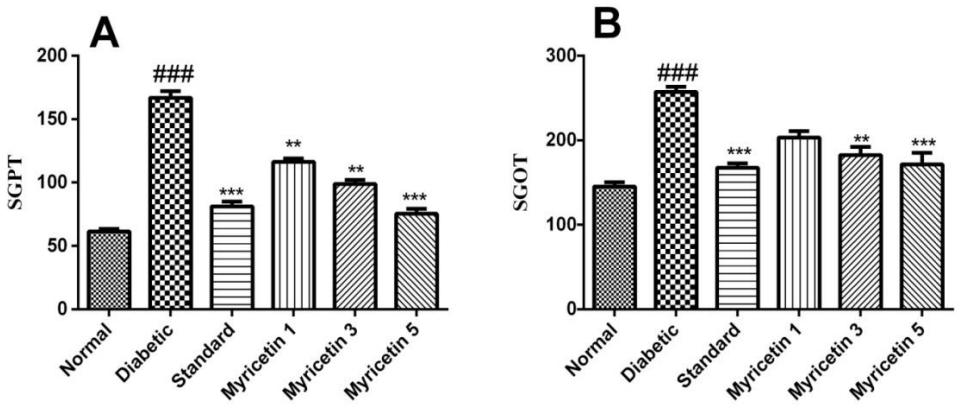

Figure 1-Effect of Myricetin on serum biochemical parameter

Data were presented as mean ± S.E.M. (n-6). The result values were statistically analysed by using one-way ANOVA and 2- way ANOVA followed by Bonferroni's test for multiple comparisn.

Effect of STZ and Myricetin on cytokines release

Release of cytokine was measured by ELISA assay and showed significant rise in TNF α and IL-6 in STZ treated rats. This was indicated by a marked increase in liver cytokine levels as compared to control rats ($p < 0.001$). Myricetin treatment at different doses in the STZ treated rats significantly restored the increased cytokine levels as compared to the STZ treated rats ($p < 0.001$) (figure 2).

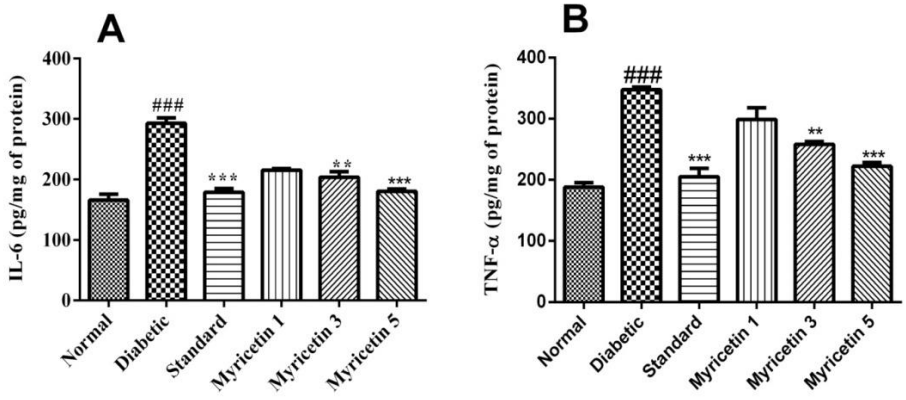

Figure 2- Effect of STZ and Myricetin on Cytokines release

Data were presented as mean ± S.E.M. (n-6). The result values were statistically analysed by using one-way ANOVA followed by Bonferroni's test for multiple comparison.

Effect of Myricetin on MDA

STZ induced diabetic rats revealed an increase in level of MDA as compared to Normal group. Oral treatment with Myricetin (1 , 3 and 5 mg/kg/day) for 4th week dose dependently decreased the level of MDA when compared with STZ induced diabetic group given in figure 3.

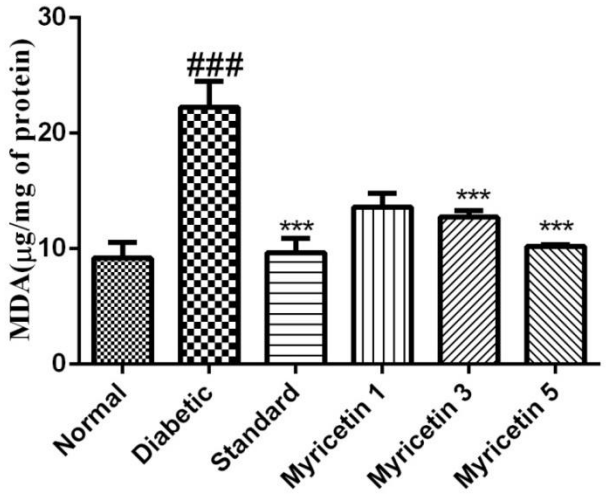

Figure 3: Effect of Myricetin on MDA

Values are expressed as means ±SEM, n = 06. Statistical significance was determined by one-way ANOVA followed by the Bonferroni's post hoc test: Compared with Normal ##P < 0.01, Compared with STZ* P < 0.05, **P <0.01.

Discussion

Oxidative stress and inflammation play crucial position in initiation and development of Diabeties. It was also recognised to enhance insulin secretion and improves glycemic manage in diabetics[13]. Hyperlipidaemia, oxidative pressure and anti-inflammatory approaches was proposed inside the pathogenesis of type 2 diabetes. Phytochemicals was known to act as powerful antioxidant and halts process of inflammation by controlling oxidative stress. Therefore, phytochemicals play significant role in control and management of diabetes mellitus and its complications[14]. Previous research had stated the function of Myricetin as hypolipidemic, anti-inflammatory and antioxidant agent. It's far recognised to set off PPAR-α and PPAR-γ receptors and inhibit the activation of NF-kB pathway. Additionally, Myricetin exhibited inhibitory effect on pancreatic β-cellular apoptosis and precipitated β-cellular regeneration[15].

In the present study, it was observed, Wistar rats were selected to create pathological capabilities just like type 2 diabetes mellitus in human. Streptozotocin changed into used to supply type 2 diabetes in experimental version. As noted previously, the severity of β-cell destruction relies upon on dose of STZ treatment. That allowed you to produce type 2 diabetes, various authors endorsed STZ dose inside the range of 25-55 mg/kg in rodent version. Hence in current study, STZ in dose of 55 mg/kg was used to induce insulin resistance and partial β-cellular disorder.

Administration of STZ significantly reduces weight of both livers in diabetic rats compared to normal rats. However, treatment with metformin and Myricetin improved weight of both liver. Similarly, weight of liver was increased in STZ administered groups and got reduced with administration of metformin and Myricetin. Results of the current study suggested that STZ in dose of 55 mg/kg induced hyperglycaemias and hyperinsulinemia in experimental animals. Treatment with Myricetin produced better glycemic control which was equivalent to standard drug metformin. Results of glucose test supported these findings. Myricetin at dose of 5 mg/kg exhibited significant improvement in fasting glucose level as compare to diabetic control group. These data suggested that Myricetin could possibly produce antidiabetic effect in diabetic rats.

STZ treatment in diabetic rats leads to increase in oxidative stress responsible for development of diabetic complications. Resemblance of pathological popularity in human diabetes with STZ caused hyperglycaemia makes it a great version for the preliminary screening of lively dealers against diabetes. Continualhy perglycemias suppress the activity of antioxidant enzymes resulting in growth in production of reactive oxygen species (ROS). ROS causes cellular damage leading to functional abnormalities in the organ. Cellular membranes and lipids were incredibly prone to the oxidative pressure and peroxide response brought on by ROS. Lipid peroxidation was traditionally an unfastened radical chain response initiated by the absorption of a hydrogen atom from a polyunsaturated fatty

acid side chain. This process was initiated via ROS main to cellular damage by using the inactivation of membrane enzymes and receptors, depolymerisation of polysaccharides, protein cross-linking and fragmentation. Additionally, lipid peroxidation leads to production of an extensive variety of cytotoxic merchandise which include aldehydes. MDA is a main marker of endogenous lipid peroxidation. In the current study, activity of MDA was significantly increased in diabetic animal as compared to normal group. However, treatment with Myricetin significantly decreased MDA activity in dose dependent manner comparable to standard drug metformin. TNF-α and IL-6 were known as a pro-inflammatory cytokine and are predictor oftype 2 diabetes. Both are thought to consider to mediate adverse metabolic effects resulting in insulin resistance and deteriorating glucose homeostasis. The level of TNF-α and IL-6 was significantly increased in STZ induced diabetic animal as compared to normal group. Treatment with Myricetin significantly decreased both and effect was equivalent to metformin.

Conclusion

Our studyprovides, the realistic proof of Myricetin for anti diabetic effect in STZ induced diabetes in rats. Antidiabetic interest elicited by means of Myricetin might be because of its capability to activate antioxidant enzymes. These study outcomes suggest that Myricetin may be used in diabetes and control of blood glucose, BUN and serum creatinine. Further studies are needed to evaluate therapeutic potential of Myricetin in diabetes in human cell line model to find the mechanism.

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