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Renoprotective effect of myricetin restrains dyslipidemia and renal mesangial cell proliferation by the suppression of sterol regulatory element binding proteins in an experimental model of diabetic nephropathy

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ABSTRACT

Myricetin is a natural flavonoid used in various health management systems. In this present study myricetin tested to evaluate the effect on lipids and lipid metabolism enzymes in normal and streptozotocin (STZ) with cadmium (Cd) induced diabetic nephrotoxic rats. Diabetic nephrotoxic rats were significantly ($P<0.05$) increased the levels of urinary albumin and lipid profiles: total cholesterol (TC), triglycerides (TGs), free fatty acids (FFAs), phospholipids (PLs), low density lipoprotein (LDL), very low-density lipoproteins (VLDL), and decreased in the levels of high-density lipoproteins (HDL). In addition, the activity of lipoprotein lipase (LPL) and lecithin cholesterol acyl transferase (LCAT) were decreased significantly, whereas the 3-hydroxy 3-methylglutaryl coenzyme A (HmgCoA) reductase activity was increased. The upregulation of sterol regulatory element binding protein-1a (SREBP-1a), SREBP-1c, SREBP-2, transforming growth factor-β_1 (TGF-β_1), vascular endothelial growth factor (VEGF) and downregulation peroxisome proliferator-activated receptor alpha (PPAR-α) proteins expression levels were noticed. An administration of myricetin (1.0 mg/kg body weight (b/w)) for 12 weeks was brought the above parameters towards normal level. Histopathological study of kidney samples showed that extracellular mesangial matrix expansion, glomerulosclerosis and interstitial fibrosis in diabetic nephrotoxic rats was suppressed by myricetin treatment. Further our results indicate that administration of myricetin afforded remarkable protection against STZ-Cd induced alterations in lipid metabolism and thereby reduced the diabetic nephropathy in experimental rats.

1. Introduction

Diabetes and diabetes-related kidney diseases are serious health problems that are the cause of growing concern in many parts of the world. It is estimated the total number of people with diabetes worldwide will rise from 171 million in 2000 to an estimated 366 million by the year 2030 (Wild et al., 2004). One of the most serious complications of diabetes is chronic kidney disease, also known as diabetic nephropathy. Diabetic nephropathy is associated with albuminuria, declining glomerular filtration rate and kidney structural changes, including thickening of the basement membranes, mesangial sclerosis, and arteriolar hyalinosis. Approximately 30–40% of type II diabetic patients have diabetic nephropathy, and it is now the most common cause of end stage renal failure in the Western world (Schrijvers et al., 2004). The impaired or altered lipid metabolism is mainly contributed to progression of diabetic nephropathy (Chen et al., 2004). Hyperlipidemia stimulates the mesangial and tubulointerstitial cells that produce membrane-bound transcription factors, cytokines and chemokines, which leads to mesangial cell proliferation via an increased production of TGF-β_1 and VEGF which promotes extra cellular matrix components deposition in the mesangium and the tubulointerstitium which ultimately leads to renal failure.

Cadmium (Cd) is a prominent environmental pollutant also nephrotoxicant that directly affects the various organ of the body mainly kidney. Intracellular damage caused by Cd exposure includes protein denaturation, lipid peroxidation, generation of reactive oxygen species and DNA strand breaks (Lopez et al., 2006). When a threshold concentration of 150–200 μg/g tissue is reached, it causes a generalized dysfunction of the proximal tubule characterized by polyuria, low molecular weight proteinuria and glucosuria (Satarug and Moore, 2004). Streptozotocin (STZ) is

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a N-methyl-N-nitrosourea derivative, which is a glucose analog, is now widely used for chemical induction of diabetes in laboratory animals as the pathological alteration is similar to that occurs in humans (Bell and Hye, 1983). STZ-Cd induced diabetic nephropathy model, consistent hyperglycemia results in increased urinary albumin excretion as well as characteristic histological changes, such as glomerulosclerosis, excess matrix deposition, and podocyte injury (Tesch and Allen, 2007). The previous report supporting that, STZ-induced diabetic rats was given Cd (100 ppm) in drinking water for 90 days had a doubling of urinary gamma-glutamyl transpeptidase (GGT) and N-acetyl-b-glucosaminidase (NAG), protenuria and enzymuria levels as compared to the STZ alone treated diabetic and non-diabetic Cd exposed groups (Jin et al., 1999). Based on this report, STZ-Cd induced diabetic nephrotoxicity is considered as a good experimental model to find diabetic nephropathy in rats.

The goals of managing diabetic nephropathy are to optimize the control of blood glucose, reduce the effects of oxidative stress and normalize disturbances in lipid metabolism. Drug management of diabetes without associated untoward defect has also remained a challenge for conventional medical practice. Therefore, numerous studies have been carried out to evaluate natural products, including plant materials, as alternative treatments for diabetes to be used in addition to conventional treatments. Myricetin is a naturally occurring flavonol with hydroxyl substitutions at the 3, 5, 7, 3′, 4′ and 5′ positions, and commonly consumed from our diet in fruits, vegetables, tea, berries, red wine and the herb named *Abelmoschus moschatus Medic*. (Malvaceae). Accumulated data indicated that myricetin has many biological activities, such as anti-oxidant, anti-inflammatory, anti-carcinogen, and anti-viral (Ong and Khoo, 1997). The recent laboratory findings were suggested that myricetin has been act as a antihyperglycemic (Kandasamy and Ashokkumar, 2012; Kandasamy and Ashokkumar, 2014) and antioxidant agent (Kandasamy and Ashokkumar, 2013) in experimental diabetic nephrotoxic rats. In the present study, we investigated the therapeutic effects of myricetin on STZ-Cd persuaded dyslipidemia caused diabetic nephropathy and its underlying molecular mechanism.

2. Materials and methods

2.1. Animals

The whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Animal Ethical Committee of Annamalai University (Reg. no. 160/1999/CPCSEA/Proposal no. 757). The study was conducted on 24 male albino Wistar rats weighing 180–220 g, obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah medical college, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages, (six rats per cage) lined with husk, renewed every 24 h under a 12:12 h light and dark cycle at around 22 °C and had free access to tap water and pellet diet (Pranav Agro Industries Limited, Maharashtra, India). The pellet diet consists of 22.02% protein, 4.25% fat, 3.02% fiber, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% carbohydrates. It provided a metabolizable energy of 3600 kcal/kg.

2.2. Drugs and chemicals

Myricetin (HPLC purity ≥ 96%), STZ and Cd were obtained from Sigma Chemical Co (St. Louis, MO, USA). Ferric chloride, isopropanol, diphenyl carbazide and perchloric acid were purchased from Himedia, Mumbai, India. Commercial diagnostic kits were obtained from Qualigens Diagnostics (Mumbai, India) for determination of cholesterol, HDLC, and TGs. All the other chemicals used were of analytical grade.

2.3. Induction of diabetic nephrotoxicity in rats

Diabetic nephrotoxicity was induced in overnight fasted rats by a single intraperitoneal injection of a freshly prepared solution of STZ (40 mg/kg body weight (b/w)) in 0.1 M sodium citrate buffer, pH 4.5, in a volume of 1 ml/kg b/w (Siddique et al., 1987). Immediately, following these injections drinking water was given which contained Cd in dose of 100 ppm as cadmium chloride ($CdCl_2$) for 12 weeks (Jin et al., 1999). After 72 h of STZ administration, the blood samples were collected from the rat tail vein. Plasma glucose was determined and those rats with fasting glucose levels greater than 250 mg/dl were used in the present study.

2.4. Experimental design

The experimental rats were divided into four groups of six in each groups; a total of 24 rats (12 diabetic nephrotoxic surviving rats, 12 non diabetic rats) were used. The treatment was started with 1.0 mg/kg b/w of myricetin after the conformation of blood glucose level in the experimental rats. Myricetin was dissolved in normal saline solution and administered intraperitoneally once a day for a period of 12 weeks. Based on our previous dose dependent study an optimal dose of 1.0 mg/kg b/w was selected for this experiment as it was found to lower blood glucose significantly during oral glucose tolerance test (OGTT) assay in STZ-Cd induced diabetes when compared to other two doses of 0.5 mg/kg b/w and 1.5 mg/kg b/w. (Kandasamy and Ashokkumar, 2012). Jin et al. (1999) reported that STZ induced diabetic control rats treated with Cd significantly ($P < 0.05$) elevated the levels of proteins in the urine and accumulation of Cd in the tissues when compared to normal rats treated with Cd. It was demonstrated that diabetic rats are more susceptible to Cd nephrotoxicity than normal rats. Based on this report, our experimental protocol was designed as follows.

Group 1	:	Normal rats.
Group 2	:	Normal rats received myricetin (1.0 mg/kg b/w) for 12 weeks.
Group 3	:	Diabetic nephrotoxic control rats (DNC).
Group 4	:	DNC rats received myricetin (1.0 mg/kg b/w) for 12 weeks.

The end of experimental period rats were fasted overnight. The rats were killed by cervical decapitation using ketamine hydrochloride (24 mg/kg b/w., intramuscular injection) as anesthesia and blood samples were collected in test tubes containing potassium oxalate and sodium fluoride (3:1 w/w) as anticoagulant and plasma was obtained after centrifugation. It had been used for the various biochemical estimations. Tissues like liver, kidney and pancreas were excised immediately from the rats and stored in liquid nitrogen container to avoid protein degradation. For enzyme assays, liver and kidney tissues were minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 4 °C 12,000*g* for 30 min and the supernatant was collected and used for further determinations. Liver, kidney and pancreas were used for histological and molecular studies.