Research Article

Forskolin, ameliorates mitochondrial dysfunction in Streptozotocin induced diabetic nephropathy in rats

Surbhi Rana[1], Lakhwinder Singh[2]*, Sidharth Mehan[3]

[1]*Research Scholar, I. K. Gujral Punjab Technical University, Jalandhar, Punjab, India*

[2]*Head Department of Applied Science CGC College of Engineering, Landran, Punjab, India*

[3]*Associate Professor, Department of Pharamcology, ISF College of Pharmacy, Moga, Punjab, India*

Abstract

Objective: Diabetes downregulates the expression of nitric oxide and elevate the reactive oxygen species resulting in oxidative stress leads to mitochondrial dysfunction. In the present study we have examined the role of Forskolin in reduced nitric oxide and mitochondrial dysfunction, which may be responsible for the development of diabetic nephropathy. **Materials and methods:** Diabetes mellitus in rats was induced by feeding high fat diet (HFD) for 2 weeks followed by single low dose of Streptozotocin (STZ) 35mg/kg, i.p. further followed by HFD for next ten weeks. STZ administered rats exhibited apparent renal functional changes as compared to normal rats. These renal functional abnormalities were complimented with increased uric acid, liver functions and mitochondrial complexes dysfunction as assessed in terms of mitochondrial Adenosine Triphosphate (ATP) levels in Liver, Kidney and Pancreas, Alkaline Phosphatase (ALP), Succinate Dehydrogenase (SDH), Nicotinamide Adenine Dinucleotide Phosphate (NADPH), Lactate Dehydrogenase (LDH). **Results:** Two weeks treatment with Forskolin presented a marked renoprotection by significantly preventing renal abnormalities supported by biomarkers of mitochondrial and liver dysfunctions tests. **Conclusion**: Hyperglycaemia produces mitochondrial dysfunction with dysregulation between oxidative stress and nitric oxide. Diminished nitric oxide level, elevated uric acid, liver and mitochondrial dysfunction could play critical role in STZ induced mitochondrial dysfunction in diabetic nephropathy. This study provides preliminary evidences that Forskolin markedly prevented mitochondrial dysfunction and progression of nephropathy.

Keywords: Mitochondrial dysfunction, mesangial expansion, oxidative stress, Forskolin

Introduction

Diabetes is the leading cause of chronic kidney disease and end stage renal disease which is most common reason for mortality and morbidity such as cardiovascular disease and renal dysfunction, a major reason for mortality (Dregan et al., 2017). The uncontrolled diabetes mellitus often leads to renal complications. Nephropathy is chief cause of death among people having chronic diabetes mellitus (Rajavel et al., 2016). Elevated serum creatinine, blood urea nitrogen, renal collagen content and reduced level of serum nitrite/ nitrate are markers of diabetic nephropathy (Bruno et al., 2014). In addition, diabetic nephropathy characterized by glomerular hypertrophy, accumulation of extracellular matrix protein, increased basement membrane thickness, mesangial expansion, podocyte loss and vascular endothelial dysfunction progressively leading to glomerulosclerosis, tubulointerstitial fibrosis, proteinuria and dyslipidaemia (Kolset et al., 2012). The progression of diabetic nephropathy can be controlled by optimal glucose control. Recent studies have demonstrated involvement of dyslipidaemia and mitochondrial dysfunction in the development of diabetic nephropathy (Rajavel et al., 2016; Sonia et al., 2017). Dyslipidaemic condition includes hypertriglyceridemia (TG), reduced high density lipoprotein (HDL) and elevated low density lipoprotein (LDL). Dyslipidaemia plays critical role in the pathogenesis of nephropathy.

Oxidative stress is a key factor in etiology of diabetic

*Address for Corresponding Author:

Lakhwinder Singh

Head Department of Applied Science CGC College of Engineering, Landran, Punjab, India-140307

E-mail: lakhwinder.pharm@gmail.com

complications like cardiomyopathy and nephropathy (Dewanjee et al., 2006; Bhattacharya et al., 2013). However, the exact source of oxidative free radicals is yet to be recognized. Mitochondrial dysfunction, Advanced Glycation End products (AGEs) and others are believed to be the probable sources (Kikkawa, 2000). It is well documented that disturbance in mitochondrial bioenergetics may be important in the development and progression of diabetic nephropathy (Forbes et al., 2008; Sivitz, 2010; Sharma et al., 2013). Generation of oxidative free radicals results in oxidative disruption of structural proteins and degradation of membrane-bound-phospholipids (Dewanjee et al., 2013). Moreover, oxidative stress also leads to impairment of endogenous antioxidant enzymes due to their non-enzymatic glycosylation and auto-oxidation (Ayalasomayajula, 2005). It is reported that inflammatory mediators play key roles in oxidative stress and insulin resistance thereby participate directly in diabetic nephropathy (Bhattacharya et al., 2013). Although, a number of therapeutic interventions like antidiabetic, antihypertensive and antioxidant drugs are available to treat diabetic nephropathy (Balakumar et al., 2009). Suggesting that the central pathogenic mechanisms involved in the initiation and progression of diabetic nephropathy are still remaining active and unmodified by the present therapies. So, there is an utmost importance of elucidating key pathogenic mechanisms of diabetic nephropathy that may pave a way for the identification of certain molecular targets for the development of effective therapeutic strategies. Recently, it has been reported that inflammatory pathways play a major role in the pathophysiology of diabetic nephropathy (Navarro et al., 2011).

Forskolin (FSK) is a diterpene derived from plant *Coleus forskohlii* (Bhat et al., 1983; Insel et al., 2003). FSK directly activates Adenylate Cyclase (AC), which increases intracellular cyclic Adenosine Mono Phosphate (cAMP) levels. Furthermore, it has been reported that Adenosine Monophosphate Activated Protein Kinase (AMPK) may be a key factor that regulate lipid metabolism, antioxidant, anti-inflammatory activity and inhibits Tumor Necrosis Factor (TNF)-α, 1β, 6 and 8 (Loft et al., 1983; Hayashida et al., 2011). The activation of the cAMP-dependent Protein Kinase (PKA) may inhibit Tumor necrosis factor-α (TNF-α) and Nuclear factor-kappa B (NF-κB) which is implicated in inflammation & oxidative stress. NF-κB is produced by almost all cell types and is activated by a wide variety of cell-stress stimuli including hyperglycemia, obesity, increased plasma free fatty acids, oxidative stress, hypertension, proteinuria and renal fibrosis etc. (Karin, 2005; Frangioudakis, 2008; Lee, 2010; Soetikno et al., 2011; Wada, 2013). The present study aimed to investigate the ameliorative effects of FSK on mitochondrial dysfunction in HFD and STZ induced diabetic nephropathy in rats.

Materials and methods

Animals and interventions

Healthy male Wistar rats weighing 180-220g were used in the present study. All rats were procured from Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana and maintained in standard light/dark cycle with free access to standard HFD and tap water ad libitum. This study was approved by Institutional Animal Ethical Committee (IAEC) (RITS/IAEC/2016/08/08) as per the instructions of CPCSEA, Government of India (888/PO/Re/S/05/CPCSEA).

Experimental Design (n = 7)

Experimental diabetes mellitus was induced by feeding HFD (normal pellet diet 365g, lard 310g, casein 250g, cholesterol 10g, vitamin and mineral mix 60g, DL-methionine 0.3g) to rats for 2 weeks followed by low dose of STZ 35mg/kg, i.p. further HFD was continued till the end of study (Srinivasan et al., 2005). Successful induction of diabetes was confirmed by assessing the blood glucose level >200mg/dl after 1 week of STZ administration. Serum glucose level was determined by glucose oxidase-peroxidase (GOD-POD) method. Diabetic rats were divided into ten different groups (n=7) for further treatment with test and standard drugs. After eight weeks of STZ administration, two weeks treatment with FSK three doses (10mg/kg, 20mg/kg and 30mg/kg) procured from Bangladesh Petroleum Exploration and Production Company Ltd., Rajasthan, India, was dissolved in distilled water and administered orally by gastric tube. Three different doses of FSK were selected on the basis of acute oral toxicity studies reported in addition to the previous studies carried out on the FSK (Sujata et al., 1983). Glibenclamide (0.6mg/kg) and Atorvastatin (0.5mg/kg/day) were obtained from Sigma Aldrich [P] Ltd., Bangalore, was dissolved in distilled water and administered orally. All other chemicals used in the present study were of analytical grade. Glibenclamide and Atorvastatin are well reported in basic and clinical studies (Akiko et al., 2015; Seena et al., 2017). Therefore both the drugs have been used as standard drugs in the present study.

Animal grouping (n=7)

Group-I (Normal control): Rats were maintained on standard food and water regimen and no treatment was given.

Group-II (FSK *per se*): Rats were administered high dose of FSK (30mg/kg, p.o.) for two weeks.

Group-III (Diabetic Control): Normal rats were fed HFD for 2 weeks, followed by single low dose of STZ (35 mg/kg,

i.p.) and further followed by HFD for another 10 weeks.

Group-IV (FSK-10mg/kg treated group): Diabetic rats were treated with FSK [10mg/kg, p.o.] for two weeks after 8 weeks of STZ administration.

Group-V (FSK-20mg/kg treated group): Diabetic rats were treated with FSK [20mg/kg, p.o.] for two weeks after 8 weeks of STZ administration.

Group-VI (FSK-30mg/kg treated group): Diabetic rats were treated with FSK [30mg/kg, p.o.] for two weeks after 8 weeks of STZ administration.

Group-VII (Glibenclamide-0.6mg/kg): Diabetic rats were treated with Glibenclamide [0.6mg/kg, p.o.] for two weeks after 8 weeks of STZ administration.

Group-VIII (Atorvastatin-0.5mg/kg): Diabetic rats were treated with Atorvastatin [0.5mg/kg/day, p.o.] for two weeks after 8 weeks of STZ administration.

Group-IX (Glibenclamide-0.6mg/kg + FSK-30mg/kg): Diabetic rats were treated with Glibenclamide [0.6mg/kg, p.o.] in combination with FSK [30 mg/kg, p.o] for two weeks after 8 weeks of STZ administration.

Group-X (Atorvastatin-0.5mg/kg + FSK-30mg/kg): Diabetic rats were treated with Atorvastatin [0.5mg/kg, p.o.] in combination with FSK [30 mg/kg, p.o] for two weeks after 8 weeks of STZ administration.

Measurement of biochemical and morphological changes

All biomarkers of diabetic nephropathy (serum glucose, blood urea nitrogen, protein in urine estimation, urine volume, serum creatinine, absolute kidney weight and kidney weight/body weight and renal collagen content) were assessed and reported earlier. However, in the present study successful development of diabetic nephropathy and mitochondrial enzymatic activity was evaluated in rats by biochemical examination.

In addition, serum nitrite/nitrate level, liver function parameters, uric acid estimation, mitochondrial respiratory enzyme complexes Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH) and Mitochondrial ATP level in kidney, liver and pancreas were accessed.

All biochemical estimations were performed using commercially available kits (Transasia Bio-Medicals Ltd., Baddi, India).

Preparation of kidney homogenate

Immediately after sacrifice, both the kidneys were dissected and rinsed with ice cold isotonic saline and weighed. The kidney was than minced and a homogenate (10% w/v) was prepared in chilled 1.15% KCL. The homogenate was used for the

Mitochondrial ATP level and other mitochondrial enzymatic estimations.

Measurement of Serum parameters

For determination of serum nitrite and nitrate assay *Sastry et al., 2002* (Sastry et al., 2002) method was followed, liver function (Serum Glutamate Pyruvate Transaminase [SGPT], Serum Glutamate Oxaloacetate Transaminase [SGOT], Alkaline Phosphatase [ALP]) and uric acid was estimated in serum samples of diabetic rats. The estimation of liver function tests and uric acid determination was carried out using biochemical kits (Transasia Bio-Medicals Ltd., Baddi, India).

Measurement of Mitochondrial Respiratory Enzyme Complexes NADPH, SDH and LDH

Measurement of NADPH Activity (Complex-I)

Kidney was homogenized mechanically in 10mM Tris-HCl pH 7.2, 225mM mannitol, 75mM saccharose and 0.1mM EDTA and centrifuged (600g, 4°C, 20 min) to obtain the post-nuclear supernatant. Complex-I activity was measured spectrophotometrically at 37°C during 3 min by the rate of NADH oxidation at 340nm in an assay medium containing 40μg protein of post-nuclear supernantant in 1mL 25mM phosphate buffer pH 7.5, 2.5mg/mL bovine serum albumin, 100μM decylubiquinone and 200μM NADH. Reactions were performed in the absence and the presence of 2μM rotenone, and the rotenone-sensitive activity was attributed to complex-I (Gunter et al., 2003).

Measurement of SDH Activity (Complex-II)

SDH is a biochemical indicator of mitochondrial dysfunction in real disease. The effective evaluation of SDH in the kidney was confirmed according to the procedure (Kumar et al., 2007). Sodium succinate solution 0.3mL was stirred with 50μL of remaining homogenate. The admixture was then incubated at 37°C for 10-20 minutes. Following addition of 0.1mL of p-iodonitro tetrazolium violet (INT), the admixture was again incubated for 10 minutes. In this procedure, reaction mixture was inhibited by 1mL of the mixture of ethyl acetate, ethanol and tricholoroacetic acid (5:5:1, v/v/w) and centrifuged at 15,000 rpm for 1 minute. The absorbance at 490nm was measured with a spectrophotometer.

Measurement of LDH Activity

LDH activity in rat kidney homogenate was measured using a LDH kit Transasia Bio-Medicals Ltd., Baddi, India) and it was expressed as IU/L (Maharaj et al., 2003; Choi et al., 2004).

Measurement of Adenosine Triphosphate (ATP) Synthase

activity in Kidney, Liver and Pancreas (Complex-V)

Aliquot of the homogenate was sonicated immediately in ice cold perchloric acid (0.1 N) to inactivate ATPases. After centrifugation (14,000g, 4°C, 5min), supernatant containing ATP was neutralized with 1N NaOH and stored at -80°C until analysis. ATP level in supernatant was quantified using reversed-phase high-performance liquid chromatography (RP-HPLC) (PerkinElmer Inc., Hopkinton, MA, USA). RP-HPLC determination was performed on a reversed-phase Hypersil C18 (4.6 mm×250 mm, 5μ) column (Elite, Dalian, China) attached to two LC-10ATvp pumps (Shimadzu, Kyoto, Japan), equipped with UV–Vis detector. The mobile phase was 100mM KH_2PO_4–$K_2HPO_4^-$ buffer solution (pH 6.0), the flow rate 1.2 mL/min, the column temperature 25°C and the detection wavelength 254nm. A reference solution of ATP was prepared according to dissolving standards (Sigma, St. Louis, MO, USA) (Ramanathan et al., 2012).

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Results obtained from various groups were statistically analysed by One Way - ANOVA followed by Post hoc Tukey's test. The level of $P<0.05$ was considered statistically significant.

Results

All drugs were dissolved in drinking water and administered orally in normal and diabetic rats for two weeks after eight weeks of STZ administration.

We have observed that development of diabetic nephropathy followed by HFD and low dose of STZ in Wistar rats. Diabetic nephropathy was confirmed by change in the serum glucose level, body weight, urine output, creatinine, cholesterol, Blood Urea Nitrogen, urinary protein, absolute kidney weight, kidney hypertrophy (kidney weight/body weight), renal collagen content.

Alteration in Serum Nitrite and Nitrate level

Serum Nitrite and Nitrate ratio was significantly reduced in diabetic rats when compared with normal control group. Treatment with low dose of FSK (10mg/kg) increased the serum nitrite/nitrate ratio but results obtained were not statistically significant. Intermediate dose of FSK (20mg/kg) increased the serum nitrite/nitrate ratio when compared with low dose treated rats and diabetic control group. Treatment with high dose of FSK (30mg/kg) significantly raised the level of serum nitrite/nitrate ratio when compared with diabetic control group and FSK intermediate dose (20mg/kg) treated group.

Synergistic effects were presented when FSK (30mg/kg) was co-administered with standard drugs and results were markedly significant when compared with diabetic control and standard drug treated groups (Table 1).

Alteration in liver function tests

Liver function tests (SGPT, SGOT and ALP) test results were found to be significantly elevated after 8 weeks of STZ administration. Treatment with low dose of FSK (10mg/kg) reduced the SGPT, SGOT and ALP level but results

Table 1. Effect of FSK on Serum Nitrite/Nitrate Ratio, SGPT, SGOT, ALP and Uric Acid level

Groups	Serum Nitrite/Nitrate Ratio (μM)	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Uric Acid (mg/dL)
Normal Control	11.16 ± 0.54	22.64 ± 4.40	15.76 ± 2.44	10.59 ± 1.77	5.22 ± 0.48
FSK per se	11.35 ± 0.65	25.07 ± 3.25	15.81 ± 2.72	10.79 ± 2.08	5.38 ± 0.67
Diabetic Control	3.62 ± 0.40[a]	84.40 ± 4.82[a]	55.74 ± 4.65[a]	51.01 ± 2.90[a]	12.56 ± 0.89[a]
FSK-10 in Diabetic group	4.25 ± 0.46[a]	78.29 ± 4.27[a]	51.86 ± 3.93[a]	47.57 ± 2.22[a]	11.43 ± 0.61[a]
FSK-20 in Diabetic group	5.39 ± 0.37[b]	61.92 ± 4.06[b]	43.98 ± 4.47[b]	40.83 ± 3.99[b]	9.71 ± 0.76[b]
FSK-30 in Diabetic group	7.68 ± 0.36[bc]	47.14 ± 4.87[bc]	36.07 ± 4.38[bc]	33.01 ± 2.35[bc]	7.66 ± 0.53[bc]
Glibenclamide in Diabetic group	8.86 ± 0.45[bd]	68.05 ± 4.13[b]	47.79 ± 2.84[b]	43.80 ± 3.58[b]	9.95 ± 0.80[b]
Atorvastatin in Diabetic group	4.80 ± 0.34[b]	58.65 ± 3.95[bd]	39.02 ± 4.22[bd]	39.67 ± 3.21[bd]	9.34 ± 0.75[bd]
Glibenclamide + FSK-30 in Diabetic group	9.8 ± 0.42[be]	39.20 ± 5.32[be]	28.26 ± 4.57[be]	26.74 ± 2.81[be]	7.40 ± 0.56[be]
Atorvastatin + FSK-30 in Diabetic group	7.42 ± 0.48[bf]	31.24 ± 3.44[bf]	25.34 ± 3.90[bf]	23.43 ± 2.06[bf]	6.43 ± 0.48[bf]

All values are expressed as mean ± SD (n = 7per group); [a]P<0.05 versus normal control and FSK per se group; [b]P<0.05 versus diabetic control & FSK-10mg/kg treated diabetic rats; [c]P<0.05 FSK-20mg/kg treated diabetic rats; [d]P<0.05 FSK-30mg/kg treated diabetic rats; [e]P<0.05 GB-0.6mg/kg treated diabetic rats; [f]P<0.05 vs Atorvastatin-0.5mg/kg treated diabetic rats

obtained were not statistically significant. Intermediate dose of FSK (20mg/kg) reduced the liver function tests when compared with low dose treated rats and diabetic control group. FSK (30mg/kg) significantly reduced the level of liver function test parameters when compared with diabetic control group and FSK (20mg/kg) treated group. No significant changes were observed when FSK high dose was administered to healthy rats for two weeks and compared with normal control group.

Co-administration of FSK-30mg/kg with standard drugs (Glibenclamide-0.6mg/kg and Atorvastatin-0.5mg/kg) presented prominent changes in the results when compared with rats treated with individual standard drug treatment (Table 1).

Alteration in uric acid tests

Serum uric acid level in diabetic rats was significantly increased by more than 2 folds when compared with normal control group. Treatment with low dose of FSK (10mg/kg) decreased the serum uric acid level but results obtained were not statistically significant. Intermediate dose of FSK (20mg/kg) reduced the serum uric acid when compared with low dose treated rats and diabetic control group. However, two weeks treatment with high dose of FSK showed marked reduction in serum uric acid level when compared with diabetic control group and intermediate dose treated group. FSK *per se* group presented no change in serum uric acid level as compared to normal control group.

FSK-30mg/kg when co-administered with Glibenclamide-0.6mg/kg and Atorvastatin-0.5mg/kg to evaluate the inclusive effects, than noticeable positive outcomes were observed when results were compared with Glibenclamide-0.6mg/kg and

Atorvastatin-0.5mg/kg treated rats (Table 1).

Alteration in Mitochondrial Complex (NADPH, SDH and ATP Synthase) activities

In diabetic rats mitochondrial complex I and II activities were reduced to half of activities observed in normal control group, whereas complex-V activity was evaluated and found decreased significantly in kidney, liver and pancreas. No change in the NADPH, SDH and ATP synthase activities were observed in healthy rats administered with FSK-30mg/kg when compared with normal control group. Treatment with low dose of FSK (10mg/kg) increased the mitochondrial complex I, II and V activities but results obtained were not statistically significant. Intermediate dose of FSK (20mg/kg) significantly increased the mitochondrial complex I, II and V activities when compared with low dose treated rats and diabetic control group. However, treatment with high dose of FSK significantly elevated the mitochondrial complex I, II and V activities when compared with diabetic control group.

Co-administration of FSK-30mg/kg with Glibenclamide-0.6mg/kg and Atorvastatin-0.5mg/kg showed significant improved results when compared with diabetic control group and rats treated individually with Glibenclamide-0.6mg/kg and Atorvastatin-0.5mg/kg (Table 2).

Alteration in LDH Activity

LDH activity was found to be elevated significantly in diabetic control group when compared with normal control rats. No significant change was observed when diabetic rats

Table 2. Effect of FSK on Mitochondrial Complex (NADPH, SDH Mitochondrial ATP Synthase Activities in Kidney, Liver & Pancreas) and LDH

Groups	NADPH (IU/L)	SDH (µmol/min/ml)	Mitochondrial ATP Synthase Activities (µmol/min/ml)			LDH (IU/L)
			In Kidney	In Liver	In Pancreas	
Normal Control	9.87 ± 0.83	4.99 ± 0.23	0.74 ± 0.06	0.42 ± 0.02	0.58 ± 0.02	361.0 ± 54.67
FSK per se	10.18 ± 0.84	4.74 ± 0.26	0.76 ± 0.04	0.47 ± 0.03	0.59 ± 0.03	342.40 ± 48.09
Diabetic Control	4.61 ± 0.56^a	2.34 ± 0.34^a	0.19 ± 0.04^a	0.17 ± 0.02^a	0.28 ± 0.03^a	1190 ± 77.16^a
FSK-10 in Diabetic group	4.98 ± 0.35^a	2.79 ± 0.22^a	0.24 ± 0.03^a	0.20 ± 0.03^a	0.26 ± 0.03^a	1093 ± 71.76^a
FSK-20 in Diabetic group	7.04 ± 0.47^b	3.30 ± 0.28^b	0.39 ± 0.02^b	0.31 ± 0.03^b	0.37 ± 0.04^b	928.7 ± 99.70^b
FSK-30 in Diabetic group	8.26 ± 0.73^{bc}	4.27 ± 0.34^{bc}	0.60 ± 0.03^{bc}	0.38 ± 0.02^{bc}	0.05 ± 0.03^{bc}	779.7 ± 69.10^{bc}
Glibenclamide in Diabetic group	5.08 ± 0.49^b	2.88 ± 0.24^b	0.51 ± 0.03^{bd}	0.33 ± 0.03^{bd}	0.40 ± 0.04^{bd}	1048.0 ± 58.18^b
Atorvastatin in Diabetic group	6.27 ± 0.52^{bd}	3.29 ± 0.22^{bd}	0.29 ± 0.03^b	0.21 ± 0.02^b	0.32 ± 0.02^b	925.9 ± 68.09^{bd}
Glibenclamide + FSK-30 in Diabetic group	6.30 ± 0.52^{be}	4.45 ± 0.30^{be}	0.67 ± 0.03^{be}	0.44 ± 0.03^{be}	0.57 ± 0.03^{be}	708.7 ± 60.96^{be}
Atorvastatin + FSK-30 in Diabetic group	7.46 ± 0.68^{bf}	4.78 ± 0.37^{bf}	0.60 ± 0.03^{bf}	0.40 ± 0.02^{bf}	0.45 ± 0.02^{bf}	611.7 ± 59.62^{bf}

All values are expressed as mean $\pm$ SD (n = 7per group); [a]P<0.05 versus normal control and FSK per se group; [b]P<0.05 versus diabetic control & FSK-10mg/kg treated diabetic rats; [c]P<0.05 FSK-20mg/kg treated diabetic rats; [d]P<0.05 FSK-30mg/kg treated diabetic rats; [e]P<0.05 GB-0.6mg/kg treated diabetic rats; [f]P<0.05 vs Atorvastatin-0.5mg/kg treated diabetic rats

were treated with low dose of FSK-10mg/kg. However, intermediate dose of FSK significantly reduced LDH activity when compared with diabetic control group and low dose treated rats. Further, FSK-30mg/kg *per se* group presented no changes in the LDH activity as compared to normal control group. Furthermore, treatment with FSK-30mg/kg significantly decreased the LDH activity when compared with diabetic control group.

Marked decrease in LDH activity was observed with FSK high dose administered with Glibenclamide-0.6mg/kg and Atorvastatin-0.5mg/kg as compared to diabetic control group and rats treated individually with standard drugs (Table 2).

Discussion

There is no drug to treat diabetic nephropathy. However, some phytochemicals and herbal extracts are being investigated to cure this disease and prevent it permanently. Natural phytochemicals have been shown to be an alternative and treatment strategy to improve renal dysfunction. Natural therapies can reduce oxidative stress in renal tree. Mitochondrial dysfunction has been proposed to mediate development of diabetes induced complications in various tissues including nephrons (Haider et al., 2011). Based on these findings we have investigated ameliorative effects of Adenyl Cyclase (AC) activator FSK on mitochondrial dysfunction in STZ induced diabetic nephropathy. Our results demonstrated that diabetes reduced nitric oxide level and impaired renal mitochondria respiration, which may be interpreted as diabetes induced alteration in functioning of Electron Transport Chain (ETC) and/or on oxidative phosphorylation like in other tissues such as cardiac muscles and liver tissues (Omar et al., 2015). This is in consistent with published reports demonstrating mitochondrial dysfunction in kidney of diabetes model. Administration of HFD for two weeks followed by low dose of STZ lead to significant increase in serum glucose, BUN, serum creatinine LDH activity, reduced nitric oxide level, impaired liver function test, elevation of serum uric acid and marked reduction of mitochondrial complex I, II and V activities. Results obtained in the present study are consistent with STZ induced diabetic nephropathy, renal dysfunction and biochemical changes reported in previous studies. Significant increase in body weight was observed due to HFD. However, in this study we have reported mainly mitochondrial dysfunction due to STZ and HFD in rats. In the present study marked decrease in serum nitrite/nitrate ratio was observed due to reduced activity of endothelial nitric oxide synthase activity causing increased level of oxidative stress and noted to be an index of diabetic nephropathy. A strong relation between nitric oxide and diabetic nephropathy has been reported (Bruno et al., 2014). In diabetes, production of ROS mediated by hyperglycaemia has been reported. Intragastric administration of FSK at 10mg/kg, 20mg/kg and 30mg/kg markedly reduced the oxidative stress

supported by significant increase in serum nitric oxide level and improved activity of mitochondrial complex activities. Marked improvement in liver function and uric acid level was observed in rats treated with FSK. Moreover, low dose of FSK (10mg/kg) restored the diabetic nephropathy induced various parameters but results obtained were not statistically significant when compared with diabetic control group. Further, FSK intermediate dose (20mg/kg) and high dose (30mg/kg) significantly and dose dependently improved various abnormal test parameters due to diabetic nephropathy in treated groups. However, co-administration of FSK-30mg/kg with standard drugs showed synergistic and significant results when compared with standard drug treated rats.

In our study, we confirmed SGPT, SGOT, ALP and uric acid tests in HFD and STZ administered rats to determine liver function test and observed marked increase in the results when compared with normal control group. Two weeks treatment with FSK-30mg/kg significantly reduced the SGPT, SGOT, ALP and uric acid when compared with diabetic control group. Moreover, results obtained from combination of FSK-30mg/kg with standard drugs prominently reduced the liver function tests and uric acid level.

Mitochondrial NADPH, SDH and ATP synthase activities and serum nitrite/nitrate level were significantly reduced in diabetic rats when compared with normal control rats. In the present study HFD and STZ significantly increased the LDH level. We have observed after STZ administration serum nitrite/nitrate level, mitochondrial NADPH, SDH and ATP level were decreased and SGPT, SGOT, ALP, serum uric acid and LDH activity were increased however, FSK reversed these alteration, suggesting antioxidant activity and improved mitochondrial dysfunction by restoring ATP level to maintain renal energy homeostasis. FSK has potential to scavenge free radicals and ROS and possess antioxidant activities.

Our study demonstrated that FSK has potential to prevent HFD followed by low dose of STZ associated increase in liver function tests, uric acid level, LDH activity and decrease in serum nitrite/nitrate ratio, mitochondrial NADPH, SDH and ATP synthase activities, which may subsequently played key role in preventing development of diabetic nephropathy in rats. Renal dysfunctions are related to kidney morphological changes where as FSK can reduced the risk of renal dysfunctions by preventing renal injury.

Conclusion

On the basis of our study, it may be concluded that in

diabetic nephropathy during mitochondrial dysfunction, the availability of nitric oxide gets diminished and level of oxidative stress gets increased whereas mitochondrial complex I, II and V activities were diminished significantly. The aim of the present study was to investigate the ameliorative effects of FSK on mitochondrial dysfunction in STZ induced diabetic nephropathy in rats. We have observed renoprotective, functional and dose dependent constructive effects of FSK in diabetic rats. In the present study, co-administration of FSK high dose with standard drugs showed synergistic effects and presented protective effects.

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