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Hepato and Renal Protective Effect of Phloretin on Streptozotocin Induced Diabetic Rats

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Abstract

The aim of the present study was to investigate the protective role of phloretin on liver and kidney of streptozotocin (STZ) induced diabetic rats. Phloretin was used at the dose of 25 mg and 50 mg/kg b.w on STZ-induced diabetic rats to analyse the blood glucose, serum insulin, total protein, liver function enzymes and kidney function markers. Significant reduction in the levels of insulin and total protein and elevated levels of blood glucose, kidney function markers such as urea, creatinine and uric acid and increased activities of serum enzymes like alanine amino transferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) were observed in diabetic rats. Treatment of diabetic rats with phloretin ameliorate the elevated levels of glucose, activities of liver function enzymes and kidney function markers and also restore the levels of insulin and total protein to near normal. The results of this study concluded that phloretin have hepato and renal protective effects on STZ-induced diabetic rats. The present findings indicate that phloretin could be considered as therapeutic agents that can ameliorate the progression of diabetic complications.

Keywords: Diabetes; Streptozotocin; Nephropathy; Phloretin; Hepatopathy; Hypoglycemic

Introduction

Diabetes mellitus is a chronic metabolic disease caused by lack of insulin or reduced insulin activity which leads to hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism [1]. World Health Organisation has projected that the prevalence of diabetes mellitus will be 300 million by 2025. Chronic hyperglycemia in diabetes is associated with damage of tissues, dysfunction and ultimate failure of various organs, especially in the eyes, kidneys, nerves, heart and blood vessels. Several pathogenic processes are involved in the progression of diabetes mellitus [2].

Diabetes are associated with higher incidence of liver and kidney damage due to formation of free radicals through glucose oxidation, non-enzymatic glycation of protein, decline in antioxidant defence system, increased activity of polyol pathway, activation of protein kinase C and cytokine production leads to oxidative stress which plays an important role in etiology of diabetes and its complications [3,4]. Chronic hyperglycemia, a clinical hallmark to diabetes results in severe metabolic imbalances and pathological changes in liver and kidney [5]. Liver is one of the main organs affected by diabetes and that this progressive disease may increase the risk of both chronic liver diseases and hepatocellular carcinoma [6]. As a consequence, the activities of liver damage markers including serum alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) are increased in the untreated diabetic patients [7].

Diabetic nephropathy has been recognised as one of the leading cause of end stage renal disease in the world. Nephropathy was developed in 30-40% of diabetic patients [8]. Diabetic nephropathy is characterised by structural as well as functional abnormalities [9]. Poor glycemic control and accumulation of advanced glycation end products (AGEs) play a significant role in the development of diabetic nephropathy. Furthermore, advanced glycation end products have been implicated in tissue damage associated with diabetic nephropathy [10]. The clinical and pathological hallmarks of diabetic nephropathy include urinary albumin excretion along with accumulation of extracellular matrix, thickening of basement membrane, mesangial expansion, hypertrophy and glomerular epithelial cell (podocyte) loss within the glomeruli. Patients with diabetic nephropathy have a progressive decline in glomerular function [11].

For these reasons, it is important to discover the drug not only cure diabetes but also reduce its complications and decrease the mortality rate. The treatment of diabetes with synthetic drugs is costly and chances of side effects are high such as weight gain, gastrointestinal disturbances and liver toxicity [12]. So, the antidiabetic drug discovery has shifted its focus towards natural plant sources which are having minimal or no side effects in the treatment of diabetes [13]. Plant derived phytochemicals such as flavonoids have been reported to exert beneficial effects because of their safety and efficacy [14].

Flavonoids represent a biologically active class of secondary metabolite plant compounds that constitute an important part of human diet and it is present in fruits and vegetables [15]. Flavonoids exhibit a multitude of biological activities such as antioxidant, antibacterial, anti-inflammatory, antiallergic, vasodilatory, anticarcinogenic, immune stimulating and antiviral properties [16]. Flavonoids are derived from many plants have received considerable attention due to their beneficial pharmacological and nutritional effects against various diseases, because of their antioxidant potency and their ubiquity in a wide range of commonly consumed foods of plant origin [17].

Phloretin is a dihydrochalcone derivative belongs to flavonoid found in apple and strawberries, but abundantly present in apple. Phloretin

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has number of pharmacological activities such as anti-inflammatory [18], antioxidant by protecting the skin from UV light induced damage [19], antitumour [20] and hepatoprotective [21]. Since hyperglycemia is accompanied by complications in liver and kidney. So, the present study was designed to investigate the hepato and renal protective effect of phloretin on STZ-induced diabetic rats.

Materials and Methods

Chemicals

Phloretin and streptozotocin were purchased from Sigma Chemicals Company, St. Louis, Mo. USA. All other chemicals and reagents were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India.

Animals

Male albino rats (Rattus norvegicus) with weight range between 180-200 g were used in the present investigation. They were housed in polypropylene cages over husk bedding and a 12 hour light and dark cycle was maintained throughout the experimental period. Rats were fed with commercial pellet diet and water ad libitum. All the animals used in this experiment were acclimatized to the laboratory condition for 2-week prior to the start of experiment. The rats used in this study were maintained in accordance with the guidelines of committee for the purpose of control and supervision on experimental animals (CPCSEA) and the study was approved by the institutional ethical committee (BDU/IAEC/2015/NE/42/Dt.17.03.2015), of Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

Induction of diabetes mellitus

Diabetes was induced in overnight fasted experimental rats by a single intraperitoneal injection of STZ (60 mg/kg b.w) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). After five days, blood glucose was analysed and determined the rats with fasting blood glucose greater than 250 mg/dL were used in the present study.

Experimental design

The animals were randomly divided into five groups of six animals in each group such as group I - normal control; group II - diabetic control; group III - diabetic rats treated with phloretin (25 mg/kg b.w); group IV - diabetic rats treated with phloretin (50 mg/kg b.w) and group V - diabetic rats treated with glibenclamide (600 µg/kg b.w). Phloretin and glibenclamide were administered orally using intragastric tube once in a day for 45 days. In each group the initial and final body weight of rats were recorded. After 45 days of treatment, the animals were anaesthetised and sacrificed by decapitation. Blood was collected and serum was separated for the estimation of glucose, insulin, liver and kidney function markers.

Estimation of biochemical parameters

Serum glucose was estimated by the method of Trinder [22] using a commercial kit (Sigma Diagnostics Pvt, Ltd., Baroda, India), insulin was assayed in serum by enzyme linked immuno sorbent assay (ELISA) technique [23] using a commercial kit and protein in the serum was determined by the method of Lowry et al. [24].

Determination of liver function markers

Serum aspartate aminotransferase and serum alanine aminotransferase was assayed by using the method of Reitman and Frankel [25]. The serum alkaline phosphatase activity was estimated by the method of Kind and King [26]. The activity of serum lactate dehydrogenase was measured by the method of King [27].

Determination of kidney function markers

Urea in the serum was estimated by the method of Fawcett and Scott [28], uric acid in the serum was estimated using the method described by Caraway [29] and serum creatinine was estimated by alkaline picrate method [30].

Statistical analysis

The data of this study were presented as mean ± SD for six rats in each group and the data were analysed by one way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Values are considered statistically significant at 5% level ($P \leq 0.05$).

Results

Table 1 shows the level of blood glucose and serum insulin in normal control and experimental animals. There was a significant elevation in blood glucose level and significant decrease in serum insulin level in STZ-induced diabetic rats when compared with normal rats. Administration of phloretin and glibenclamide to diabetic rats tended to bring blood glucose and serum insulin towards near normal levels.

The serum AST, ALT, ALP and LDH activities in all groups are shown in Table 2. The serum AST, ALT, ALP and LDH levels were significantly increased in diabetic group when compared to non-diabetic group. In diabetic rats treated with phloretin and glibenclamide, there was a significant decrease in the levels of liver function markers AST, ALT, ALP and LDH in serum when compared with diabetic control rats.

The effect of phloretin on the levels of protein, serum urea, uric acid and creatinine were analysed in control and experimental diabetic rats and the results were given in Table 3. Altered levels of total protein, serum urea, creatinine and uric acid were observed in diabetic rats when compared to normal control rats. The increased levels of urea, uric acid and creatinine in diabetic rats were significantly reduced after the administration of phloretin and glibenclamide to diabetic rats. The total protein level in STZ-induced diabetic rats showed significant reduction than normal control rats. The administration of phloretin and glibenclamide to diabetic rats increased the level of total protein.

Discussion

Diabetes mellitus is associated with several complications in different systems of the body and it is increasing rapidly worldwide [31]. Streptozotocin is used as investigational drug for diabetes research. For its selective pancreatic β-cells toxicity, it is recognised as diabetes inducer in experimental animal model for understanding the pathogenesis and complications of diabetes [32]. Insulin production in β-cells of pancreas is impaired by methylation of DNA by STZ resulting in provocation of nuclear enzyme poly ADP-ribose polymerase (PARP), and therefore, depletion of NAD+ and ATP occurred. Finally, intracellular metabolism of STZ produces nitric oxide that causes DNA fragmentation leading to necrosis of β-cells, thereby the rate of insulin synthesis is diminished and it resulted to a clinical condition known as hyperglycemia [33,34].

In the present investigation, the diabetic animals exhibited marked increase in glucose level and decrease in insulin level when compared to normal control. Reduction in the blood glucose and elevation in insulin level were observed in phloretin treated diabetic groups. Our

Groups	Glucose (mg/dL)	Insulin (µU/mL)
Group I Normal control	85.23 ± 5.96^{b}	53.21 ± 1.98^{c}
Group II Diabetic control	284 ± 22.68^{a}	32.25 ± 1.24^{a}
Group III Diabetic + Phloretin (25 mg/kg b.w)	98 ± 20.06^{b}	45.57 ± 1.35^{b}
Group IV Diabetic + Phloretin (50 mg/kg b.w)	90 ± 7.28^{b}	52.65 ± 1.85^{c}
Group V Diabetic + Glibenclamide (600 µg/kg b.w)	97 ± 20.77^{b}	51.74 ± 1.62^{c}

Note: Values are expressed as mean ± SD of six animals in each group. Values not sharing a common superscript letter differ significantly at 5% level (P≤0.05) using Duncan's Multiple Range Test (DMRT)

Table 1: Effect of phloretin on serum glucose and insulin.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)
Group I Normal control	38.91 ± 2.72^{c}	29.64 ± 2.08^{c}	65.67 ± 4.48^{c}	124.49 ± 8.61^{c}
Group II Diabetic control	94.37 ± 6.60^{a}	62.11± 4.13^{a}	120.59 ± 8.43^{a}	281.11 ± 19.62^{a}
Group III Diabetic + Phloretin (25 mg/kg b.w)	54.10 ± 3.64^{b}	46.06± 4.41^{b}	88.21 ± 5.73^{b}	152.73 ± 10.58^{b}
Group IV Diabetic + Phloretin (50 mg/kg b.w)	45.36 ± 3.16^{c}	34.07 ± 2.54^{c}	72.57 ± 5.04^{c}	135.13 ± 9.55^{c}
Group V Diabetic + Glibenclamide (600 µg/kg b.w)	48.52 ± 3.39^{c}	34.66 ± 2.63^{c}	83.28 ± 5.86^{b}	142.72 ± 9.16^{c}

Note: Values are expressed as mean ± SD of six animals in each group. Values not sharing a common superscript letter differ significantly at 5% level (P ≤ 0.05) using Duncan's Multiple Range Test (DMRT)

Table 2: Effect of phloretin on AST, ALT, ALP and LDH in serum.

Groups	Protein (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
Group I Normal control	7.59 ± 0.53^{c}	21.32 ± 1.49^{c}	0.89 ± 0.06^{c}	2.58 ± 0.18^{c}
Group II Diabetic control	4.68 ± 0.32^{a}	41.43 ± 2.90^{a}	1.28 ± 0.08^{a}	6.47 ± 0.45^{a}

Group III Diabetic + Phloretin (25 mg/kg b.w)	6.04 ± 0.42[b]	30.53 ± 2.13[b]	0.94 ± 0.06[b]	4.27 ± 0.29[b]
Group IV Diabetic + Phloretin (50 mg/kg b.w)	7.37 ± 0.51[c]	23.02 ± 1.61[c]	0.85 ± 0.05[c]	3.13 ± 0.16[c]
Group V Diabetic + Glibenclamide (600 µg/kg b.w)	7.41 ± 0.53[c]	28.57 ± 1.99[b]	0.90 ± 0.06[b]	3.47 ± 0.21[c]

Note: Values are expressed as mean ± SD of six animals in each group. Values not sharing a common superscript letter differ significantly at 5% level (P≤0.05) using Duncan's Multiple Range Test (DMRT)

Table 3: Effect of phloretin on protein, urea, creatinine and uric acid in serum

results are in agreement with the report of Punithavathi et al. [35], who demonstrated that gallic acid significantly reduced the blood glucose via the secretion of insulin from regenerated β-cells in STZ-induced diabetic rats. Moreover, Ambika et al. [36] and Relaxin-Shairibha et al. [37] reported that the hypoglycemic effect of p-coumaric acid in STZ induced diabetic rats. In this study, the phloretin may be due to enhancing glucose uptake on peripheral tissues and inhibiting hepatic gluconeogenesis or inhibiting the absorption of glucose in intestine via stimulation of insulin secretion.

Liver is the vital organ involving in maintaining the optimum blood glucose level within narrow limits. Hyperglycemia induced free radical toxicity causes severe liver damage [38,39]. Moreover, LDH (a marker of nonspecific cellular injury), AST (a nonspecific marker for hepatic injury) and ALT (a specific marker for hepatic parenchymal injury) are used in the evaluation of hepatic disorders [40]. An increase of these enzyme activities reflects active liver damage [41] and an increase in the activities of serum ALT, AST, ALP and LDH indicates liver dysfunction and moreover liver was necrotized in STZ-induced diabetic rats [42]. ALT and AST are transaminase enzymes that catalyse amino transfer reactions and play an important role in amino acids catabolism and biosynthesis [43,44]. In addition, ALP is a hydrolase enzyme which acts as non-specific phosphomono esterases to hydrolyse phosphate esters [45].

The present study showed that liver function markers in serum were increased in diabetic rats that agree with the findings of Abdel-Moneim et al. [46]. In the serum, the elevation of liver function markers may occur as a result of deleterious effect of hyperglycemia in the liver of diabetic rats. A rise in ALT activity indicates the hepatocellular damage followed by cardiac tissue damage and is usually accompanied by a rise in AST activity. Further, ALP is a marker of biliary function and cholestasis. An increase in the activities of AST, ALT, ALP and LDH in serum of diabetic rats might be mainly due to the leakage of these enzymes from the liver cytosol into blood stream which gives an indication of hepatic injury [47]. These results are in line with Arkkila et al. [9], they reported that elevated activities of serum AST, ALT, ALP and LDH is a common sign of liver diseases and observed frequently among people with diabetes. However, the treatment of diabetic groups with phloretin for 45 days could ameliorate the elevated activities of these enzymes. Our results are in agreement with Ali Reza et al. [21], they demonstrated that the hepatoprotective activity of phloretin and hydroxychalcones against acetaminophen induced hepatotoxicity in mice. These results suggest that a hepatoprotective role of phloretin against liver injury associated with diabetes.

Metabolic renal changes are observed in experimental diabetes, which leads to negative nitrogen balance, increased proteolysis and lowered protein synthesis [48]. Changes in protein metabolism include a reduced uptake of amino acids by tissues, a higher rate of proteolysis and a fall in protein synthesis leading to an increase in the production of urea by the liver [49]. The elevated level of urea, glucose and other compounds in the kidney arising from the increased glycation of blood proteins can produce vascular changes in the renal system and damage the kidney and thus promote a loss of protein in the urine [50].

Reduction in serum total protein in diabetic animals was observed in the present study and this corroborates in earlier reports [51]. The level of serum total protein was found to be decreased in diabetic rats may be attributed to increased muscle proteolysis, decreased amino acid uptake [52], increased conversion of glycogenic amino acid to carbon dioxide and water, reduction in protein synthesis [53] and reduction in protein absorption [54]. In the phloretin and standard drug glibenclamide treated diabetic rats, the protein level was reverted to near normal range which might be due to the stimulation of insulin and reduce the catabolism of protein.

Diabetic nephropathy is a common and serious complication where kidneys are damaged and fails to function. In the present investigation, the STZ induced diabetic rats is associated with significant increase in the levels of urea, uric acid and creatinine are indicating impaired renal function. The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine and uric acid which are considered as significant markers of renal dysfunction [55]. Urea is the end product of protein catabolism in the living system. They are synthesized in the liver from ammonia, produced as a result of the deamination of amino acids [56]. The amount of urea in blood was elevated in diabetic state as a result of increased proteolysis in blood and tissues due to negative nitrogen balance which is associated with dropping the protein synthesis [57] and also increased oxidative stress induce the elevation in the levels of urea, creatinine and uric acid [58].

Uric acid is the metabolic end product of purine catabolism. The uric acid level in blood was significantly increased in diabetic condition due to excessive production of purine and the activity of xanthine oxidase via the metabolic pathway [59]. Moreover, protein glycation in diabetes may leads to muscle wasting and increased release of purine, which is the main source of uric acid [60]. In this study, the levels of urea and uric acid were increased in STZ-induced diabetic rats when

compared to normal control rats. Phloretin and glibenclamide treated diabetic rats showed near normal level of urea and uric acid. In the previous report, the researchers observed that the decreased levels of urea and uric acid in STZ induced diabetic rats treated with gallic acid [61], which are consistent with the present study.

Serum creatinine concentration is used as biochemical diagnostic markers to assess the impairment of renal function and drug-induced toxicity in clinical practice [62]. Creatinine is a derivative of creatine and phosphocreatine, these are considered as an energy storage compounds in muscle. The variable concentration of creatinine is not only used to evaluate the deficiency of kidney function, whereas used to detect and the treatment of associated toxic properties of compounds/drugs in the kidney of experimental rats [63]. In the present investigation there is a significant elevation in the levels of creatinine in STZ-induced diabetic rats and these results are in agreement with those of Wilson et al. [64] and Nasry et al. [65] in that they suggested the phenolic acids, sinapic acid and caffeic acid showed the same effects on kidney function parameters. In the present study, the treatment of phloretin and glibenclamide to diabetic rats reversed the altered levels of serum urea, creatinine and uric acid to near normal, which indicates the renoprotective role of phloretin in diabetic nephropathy.

Conclusion

The result of the present study indicates that the phloretin posses protective effects on liver and renal injury induced by STZ. The hepato and renoprotective effect of phloretin is demonstrated by the reduction in liver function enzymes and kidney function markers in the serum of diabetic treated rats. From this study, we concluded that phloretin is a potent hypoglycemic agent that can prevent the development of diabetic complications such as hepatopathy and nephropathy.

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