PROTECTIVE EFFECT OF CHLOROGENIC ACID AGAINST DIABETIC NEPHROPATHY IN HIGH FAT DIET/STREPTOZOTOCIN INDUCED TYPE-2 DIABETIC RATS.

*NISHI, 2AMJID AHAD, 3PAWAN KUMAR*

1Department of Biochemistry, Faculty of Life Sciences, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, 2Department of Biochemistry, Faculty of Science, Jamia Hamdard, New Delhi, India, 3Faculty of Science, Govt. P.G. College, Narnaul, Haryana, India.

Email: drpawankumar1988@gmail.com

Received: 18 Mar 2013, Revised and Accepted: 21 Apr 2013

ABSTRACT

Objective: Diabetic nephropathy (DN) is one of the leading causes of death in diabetic patients. Treatment of DN is one of the major approaches towards decelerating the diabetes related complications. The present study was designed to investigate if chlorogenic acid (CGA) isolated from *Coffee canephora* could improve the renal function in Diabetic nephropathy (DN) by the virtue of its hypoglycemic and antioxidant activities.

Methods: Male Wistar rats were randomly divided into four equal groups including normal healthy rats, diabetic untreated rats and two groups of diabetic rats treated with chlorogenic acid. Type-2 diabetes was induced by feeding the rats with high fat diet initially for 6 weeks followed by single intraperitoneal injection of streptozotocin (STZ) (35 mg/kg BW). CGA was supplied to the rats orally at two doses, 10 mg/kg BW/day and 20 mg/kg BW/day respectively over a period of 10 weeks post-induction of diabetes. After 10 weeks of STZ injection and CGA treatment, 24 h urine was collected and animals were sacrificed. Fasting blood glucose, glycosylated hemoglobin, biochemical markers of renal function and oxidative stress were evaluated in serum, urine and kidney tissue. Also the biochemical findings were matched with histopathological verification.

Results: The results showed that treatment with CGA-10 mg/kg BW/day as well as CGA-20 mg/kg BW/day significantly improved the glycemic status and renal function in Type-2 diabetic rats but CGA-20 mg/kg BW/day afforded greater efficiency as compared to CGA-10 mg/kg BW/day.

Conclusion: The present investigation suggests that chlorogenic acid can be used as an alternative treatment against DN.

Keywords: Alternative therapy, Chronic disease, Diabetes mellitus, Therapeutics.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease in which the body does not produce or/and utilize insulin [1]. DM is the third leading cause of death across the world. Approximately, 176 million individuals had diabetes in 2000 and the number is expected to increase up to 376 million by 2030 [2]. DM is of mainly two types, Type-1 and Type-2 DM which are caused by a complex interaction of genetics and environmental factors [3]. Type-2 DM is one of the common types of diabetes in world and is increasing in present time [4]. Type-2 DM is characterized by chronic hyperglycemia and development of diabetes specific microvascular complications in retina, kidney and nervous tissue. As a consequence of its microvascular pathology, diabetes is the leading cause of blindness, end-stage renal disease and a variety of neuropathies [5]. Diabetic nephropathy (DN) is a major cause of illness and death in diabetic patients [6]. About 30% of type 2 diabetic patients develop nephropathy [7], which is characterized by persistent proteinuria, progressive reduction of glomerular filtration rate (GFR) and increased morbidity and mortality due to cardiovascular diseases [8,9]. Long term diabetes and poor glycemic control have been found to be important risk factors for the development of DN [10]. Although the pathogenetic mechanism of Type-2 DM is not fully determined, however disorder in management of glucose and lipids has been demonstrated as one of the major risk factors of this disease [11]. It has also been reported that diabetic patients exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby depleting the activity of antioxidative defense system and promoting the generation of free radicals [12]. Reactive oxygen species may directly oxidize and damage DNA, proteins and lipids and are believed to play a key role in the pathogenesis of DN [13].

A multitude of medicinal plants and the active compounds obtained from them have been studied for the treatment of diabetes and its related complications all over the world as they might provide a basis of new synthetic anti-diabetic analogues with potent activity [14]. Presently, there is growing interest in herbal remedies and alternative therapies due to the side-effects associated with conventional therapeutic agents for the treatment of Type-2 DM.

Chlorogenic acid (CGA) is a phenolic compound, a family of naturally occurring organic compounds found in plants. It is present in high quantity in coffee (*Coffee canephora*). It is an ester from of cinnamic acid and quinic acid and is also known as 5-o-cafeoylquinic acid (5-CQA) [15]. Pharmacologically, CGA has been reported to delay glucose absorption in the intestine through inhibition of glucose-6-phosphate translocase [16]. It has also been reported to possess antioxidant activity and antiatherosclerotic activity [17, 18]. The aim of the present study was to investigate if chlorogenic acid (CGA) could improve the renal function in DN by the virtue of its hypoglycemic and antioxidant activities.

MATERIALS AND METHODS

Chemicals and reagents
Chlorogenic acid (CGA), streptozotocin (STZ), sulphamamide, N-(1-Naphthyl)-ethylene diamine-dihydrochloride (NED) and pyrogallol were purchased from Sigma Aldrich chemicals Pvt Ltd. (New Delhi, India). Methanol, ethanol, hydrogen peroxide, Tris-buffer, Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from SD-Fine Pvt. Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

Animals
Adult male albino rats of Wistar strain weighing 200-250 g were used throughout the study. The animals had free access to food and water ad-libitum. The animals were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating 12hr light and dark cycle. The animals were maintained in accordance with guidelines prescribed by national Institute of Nutrition, Indian Council of Medical Research and the study was approved by the animal ethics committee of Singhania University, Jhunjhunu, Rajasthan.

Experimental Design
Rats were given high fat diet (HFD) (60% fat, 18% protein and 21% carbohydrate) ad-libitum, for the initial period of six weeks [19]. After 6 weeks of dietary manipulation, animals were injected intraperitoneally with a low dose of STZ (35 mg/kg BW dissolved in
normal saline). 7 days post STZ induction, blood was collected through tail vein after 12 hour fasting and blood glucose concentration measured by a strip-operated glucometer (Accu Chek, Roche Diagnostics Pvt. Ltd., New Delhi, India). Animals with fasting blood glucose (FBG) concentration ≥ 250 mg dL-1 were considered diabetic and included in the study. Diabetic rats were divided into three groups of six each. First group served as diabetic control group and received only normal saline. The other two groups received oral administration of chlorogenic acid (CGA) at dose levels of 10 mg/kg BW and 20 mg/kg BW per day respectively for a period of 10 weeks. Six normal age matched rats were also included in a separate group that served as normal control group.

Sample collection and tissue preparation
After 10 weeks, rats were housed individually in metabolic cages for 24 hours. The urine samples were collected over a period of 24 hours. Post-collection, the urinary volume was measured. Prior to sacrifice, FBG was determined. The blood was collected from the retroorbital plexus with the help of hematoerot capillaries (SD-Fine Pvt. Ltd., India) and the animals were sacrificed by cervical dislocation under light ether anaesthesia. The animals were perfused transcardially with normal saline. Left kidney was removed and immediately preserved in 10% buffered formalin solution for histopathological examination. Right kidney was removed and washed with phosphate buffer and then homogenized in a homogenizing buffer (0.1 M phosphate buffer, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 9,000 g for 20 minutes to remove debris. The supernatant was further centrifuged at 15,000 g for 20 minutes at 40°C to get post mitochondrial supernatant (PMS) which was used for various biochemical assays. Protein concentration was estimated in PMS by the method of Bradford et al. [20].

Determination of FBG concentration and % glycosylated hemoglobin (%HbA1c)
FBG concentration was determined in blood samples obtained at the end of 10-week study period using strip operated glucometer (Accu Chek, Roche Diagnostics Pvt. Ltd., New Delhi, India). %HbA1c was determined in EDTA-blood samples after 10 week study period using commercial assay kit from CREST bio systems (Coral Clinical Systems, Mumbai, India).

Evaluation of Kidney function
Creatinine clearance was determined in order to estimate glomerular filtration rate (GFR). Creatinine was measured in serum and urine using commercial assay kit from Span Diagnostics Pvt. Ltd. (Surat, India). Creatinine clearance was calculated by the standard equation [21] Total protein was measured in urine by the Bradford method [20] using BSA as standard. BUN levels were measured in serum samples by using commercial assay kit from Span Diagnostics Pvt. Ltd. (Surat, India).

Evaluation of antioxidant status
Estimation of lipid peroxidation
Lipid peroxidation in kidney was estimated by the formation of Thioarbituric acid reactive substances (TBARS) by the method of Niehues and Samuelson [22]. In brief, 0.1 ml of tissue homogenate (PMS; 0.1 M phosphate buffer, pH 7.4) treated with 2 ml of a (1:1 ratio) TBA: TCA: HCl reagent (0.37 % TFA, 0.25 N HCl and 15% TCA) placed in boiling water bath for 15 minutes, cooled and centrifuged at room temperature for 10 minutes. The absorbance of the clear supernatant was measured against reference blank at 535 nm in a spectrophotometer (Shimadzu-1601, Japan). The TBARS content was calculated by using a molar extinction coefficient of 1.56 × 105 M-1 cm-1 and expressed as nmol TBARS formed min-1 mg-1 protein.

Superoxide dismutase (SOD) activity
Superoxide dismutase (SOD) activity was measured according to the method of Marklund and Marklund with minor modifications [23]. Briefly, the assay mixture consisted of 2.875 ml of Tris-HCl buffer (50 mM, pH 8.5), 25 μl of pyrogalol (24 mM in 10mM HCl) and 100 μl of PMS in a total volume of 3 ml SOD activity was measured at 420 nm in a spectrophotometer (Shimadzu-1601, Japan) and expressed as Units mg-1 protein. One Unit of enzyme represents the enzyme activity that inhibits auto-oxidation of pyrogalol by 50%.

Catalase activity
Catalase activity was measured by Claborne method [24]. Briefly, the assay mixture consisted of 1.9 ml phosphate buffer (0.05 M, pH 7.0); 1 ml of hydrogen peroxide (0.019 M) and 0.1 ml of PMS in a total volume of 3 ml changes in absorbance were recorded at 240 nm. Catalase activity was expressed as nmol H2O2 consumed min-1 mg-1 protein.

Kidney histopathological examination and western blotting for renal Transforming growth factor (TGF-β)
Small pieces of kidney tissue with cortex and medulla were fixed in 10% formalin and embedded in paraffin. Four micron sections were cut and stained in hematoxylin-eosin stain. Abnormality in kidney tissue was analyzed using an image-analyzing system (B × 51 Universal Research microscopes, Olympus, Tokyo, Japan) at a magnification of 10x.

Total protein quantification of whole kidney homogenate was performed using Bradford assay method (Bradford, 1976) using BSA as a standard. Samples were reconstituted in sample buffer and denatured by boiling for 5 min. Samples containing 100 μg of protein were loaded on SDS-PAGE Unit (Bio-Rad, California, USA) containing 5% stacking and 15% resolving polyacrylamide gels. Molecular weight standards (Bio-Rad, California, USA) were used to determine the molecular weights of various proteins in each sample. For western blotting, proteins were transferred to polyvinylidene fluoride (PVDF) membranes (0.45 μm) (Bio-Rad, California, USA).

Non-specific protein binding was blocked by incubation with 3% BSA in Tris-buffered saline (TBS) at 37°C for 2 hours. Membranes were incubated with rat polyclonal antibodies against TGF-β (1:150, Sigma Aldrich, USA), and β-actin (1:100, Zymed Laboratories, Inc., USA) for 16 hours at 4°C. Thereafter, blots were washed and then incubated with peroxidise-conjugated secondary antibody (Monoclonal anti- rat IgG peroxidise antibody; 1:3000, Sigma Chemicals Co, USA) for 1h at room temperature. β-actin was used as loading control. The membrane was then washed and thereafter incubated with the substrate solution (0.05% DAB + 3% H2O2+ H2O) at room temperature for few minutes. The reaction was stopped by agitating the membrane with distilled water. Protein band density was quantified by transmission densitometry (VVP Bio-imaging Systems software, Cambridge, UK).

Statistical Analysis
Data were expressed as mean ± standard deviation (SD). For the statistical analysis of the data, experimental groups were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The Tukey Kramer post hoc test was applied to identify significance among groups. p≤ 0.001 was considered to be statistically significant. Graph Pad Instar V3 (San Diego, USA) was used for statistical analysis.

RESULTS
FBG and %HbA1c
Both FBG concentration and %HbA1c level was found to be significantly (p<0.001) increased in diabetic rats as compared to normal rats when left untreated for 10 weeks (Table 1 and Figure 1). Administration of GCA for 10 weeks significantly (p<0.001) reduced levels of FBG and HbA1c % in diabetic treated rats as compared to diabetic untreated rats. Although, both the dose levels of GCA significantly ameliorated the concentration of FBG and HbA1c %, however, the GCA dose level of 20 mg/kg BW was found to be more effective.

Kidney function assays
Table 1 shows the serum creatinine level in different groups of rats. Serum creatinine was found to be increased significantly (p<0.001) in diabetic untreated rats as compared to normal rats. Chorogenic
acid treatment reduced the elevated level of serum creatinine in diabetic rats significantly (p<0.001) as compared to diabetic untreated rats. Creatinine clearance (urinary creatinine) was found to be decreased significantly (p<0.001) in diabetic rats as compared to normal rats (Figure 2). Treatment of chlorogenic acid for 10 weeks at dose levels of 10 mg/kg BW and 20 mg/kg BW elevated the reduced level of creatinine clearance in diabetic rats. The dose level of 20 mg/kg BW showed the potent activity.

Table 1: Effect of CGA Treatment on FBG, Serum Creatinine and BUN against HFD/STZ-induced Diabetic Nephropathy in Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>DC</th>
<th>CGA-10</th>
<th>CGA-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>86±3.6</td>
<td>325±5.7*</td>
<td>153±4.5**</td>
<td>114±3.6**</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>0.65±0.04</td>
<td>1.37±0.06*</td>
<td>0.92±0.06**</td>
<td>0.76±0.07**</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>6.27±0.25</td>
<td>35.6±0.62*</td>
<td>17.5±0.31**</td>
<td>11.7±0.24**</td>
</tr>
</tbody>
</table>

NC: Normal control; DC: diabetic control; CGA-10: diabetic rats treated with CGA (10 mg/kg BW); CGA-20: diabetic rats treated with CGA (20 mg/kg BW). Data are represented as mean ± S.D.

* P < 0.001 compared with normal control; ** P < 0.001 compared with diabetic control.

Fig. 1: Effect of CGA on %HbA1c in STZ-induced diabetic rats.

NC: Normal control; DC: diabetic control; CGA-10: diabetic rats treated with CGA (10 mg/kg BW); CGA-20: diabetic rats treated with CGA (20 mg/kg BW). Values are shown as mean ± S.D.

*P < 0.001 compared with normal control; **P < 0.001 compared with diabetic control.

Fig. 2: Effect of CGA on creatinine clearance in STZ-induced diabetic rats.

NC: Normal control; DC: diabetic control; CGA-10: diabetic rats treated with CGA (10 mg/kg BW); CGA-20: diabetic rats treated with CGA (20 mg/kg BW). Values are shown as mean ± S.D.

*P < 0.001 compared with normal control; **P < 0.001 compared with diabetic control.
Diabetic rats showed increased level of proteins in their urine as compared to normal rats (p<0.001) (Figure 3). Chlorogenic acid treatment reduced the increased level of proteinuria in diabetic rats. CGA dose level of 20 mg/kg BW was found to be more effective than dose level of 10 mg/kg BW.

Table 1 shows the BUN levels in different groups of rats. Serum level of BUN was found to be increased significantly (p<0.001) in diabetic rats as compared to normal rats when left untreated for 10 weeks. Treatment with chlorogenic acid significantly (p<0.001) diminished the increased level of BUN in rats when compared with diabetic untreated rats.

Renal antioxidant status parameters

TBARS content was elevated significantly (p<0.001) and SOD and catalase activities were decreased in kidney of diabetic rats as compared to normal rats when left untreated for 10 weeks (Table 2). Treatment with chlorogenic acid at dose levels of 10 mg/kg BW and 20 mg/kg BW significantly (p<0.001) decreased the increased level of TBARS and significantly (p<0.001) increased the decreased activities of SOD and catalase (Table 2). Although both the dose levels of chlorogenic acid restored the antioxidant status close to the normal values, however, 20 mg/kg BW dose level was found to be more effective.

Table 2: Effect of CGA Treatment on Renal Antioxidant Parameters against HFD/STZ-Induced Diabetic Nephropathy in Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>DC</th>
<th>CGA-10</th>
<th>CGA-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol TBARS formed min⁻¹ mg⁻¹ protein)</td>
<td>1.05±0.06</td>
<td>2.82±0.07*</td>
<td>1.53±0.05**</td>
<td>1.34±0.07***</td>
</tr>
<tr>
<td>SOD (units mg⁻¹ protein)</td>
<td>9.52±0.84</td>
<td>3.67±0.56*</td>
<td>6.32±0.63**</td>
<td>7.26±0.72**</td>
</tr>
<tr>
<td>Catalase (nmol H₂O₂ consumed min⁻¹ mg⁻¹ protein)</td>
<td>4.28±0.28</td>
<td>1.16±0.17*</td>
<td>2.95±0.23**</td>
<td>3.47±0.26**</td>
</tr>
</tbody>
</table>

NC: Normal control; DC: diabetic control; CGA-10: diabetic rats treated with CGA (10 mg/kg BW); CGA-20: diabetic rats treated with CGA (20 mg/kg BW).

Data are represented as mean ± S.D.

*P < 0.001 compared with normal control; **P < 0.001 compared with diabetic control.
Histopathological Findings

The diabetic rats (Figure 4b) after 10 weeks developed pathological changes in the glomerulus with tubular injury as compared to normal rats (Figure 4a). Pharmacological treatment with chlorogenic acid (10 mg/kg BW i.v. and 20 mg/kg BW i.v., 10 weeks) (Figure 4c and 4d) prevented the diabetes-induced pathological changes in kidney by improving tubular and glomerular injury in diabetic rats. However, the glomerular damage was less prominent in group CGA-20 (receiving dose level of 20 mg/kg BW) compared to group CGA-10 (receiving dose level of 10 mg/kg BW).

Western blot analysis of protein expression of TGF-β

Immunoblot analyses demonstrated that the protein level of TGF-β (Fig. 5) was increased significantly in DC group as compared to the normal control group. Treatment with 10 mg and 20 mg CGA/kg BW/day for 10 weeks post induction of diabetes, significantly (P<0.001) lowered the levels of TGF-β. The expression levels of TGF-β in CGA-20 group rats were markedly reduced to near basal levels as seen in the control group.

DISCUSSION

Type-2 DM is one of the most common disorders of carbohydrate metabolism. It is a life-long disease and is associated with multiple metabolic complications including obesity, hypertension and hyperlipidemia. With the progression of type2 DM, various microvascular and macrovascular complications arise. Diabetic nephropathy is one of those microvascular complications of diabetes which leads to end stage renal disease. The present study was designed to evaluate the protective role of chlorogenic acid against diabetic nephropathy in high fat diet and STZ induced type 2 diabetic rats. STZ induced diabetic rats represent a good experimental model of diabetes with minimum or no insulin production by pancreatic β-cells.

Obesity has been regarded as one of the major risk factors for type 2 DM [25]. High fat diet was administered to the rats in order to make them obese so as to increase the chances of insulin resistance [26]. STZ is an antibiotic obtained from Streptomyces achromogenes. STZ enters the pancreatic cells and causes alkylation of DNA leading to pancreatic damage which leads to decreased synthesis/release of insulin further adding to diabetes [27].

Elevated fasting blood glucose concentration is an important index of diabetes mellitus. In diabetic control rats, FBG concentration was elevated as compared to normal rats. Chlorogenic acid administration resulted in reduction of elevated blood glucose concentration in diabetic rats. The possible mechanism may be the uptake of blood glucose by cells through the increased expression of GLUT-4 or the decrease in the absorption of glucose from intestine [28]. In uncontrolled diabetes, there is an increased glycosylation of a number of proteins including haemoglobin [29]. It has been found that HbA1c increases in diabetic patients and the amount of increase is directly proportional to fasting blood glucose concentration [30]. HbA1c is considered as a very sensitive index for glycemic control. Diabetic control rats showed a significant increase in the level of HbA1c as compared to normal rats. Treatment with CGA showed a significant decrease in the glycosylated hemoglobin levels, which could be due to improvement in glucose utilization.

It has been reported that hyperglycemia leads to an increased production of glomerular matrix proteins leading to their accumulation and decrease in surface area of glomerulus which in turn decreases the glomerular filtration rate (GFR) [31, 32]. Serum and urinary creatinine and BUN measurement is taken as an index of altered GFR in diabetic nephropathy [33]. Our results showed that the level of serum creatinine and BUN was significantly elevated whereas creatinine clearance was significantly reduced in diabetic untreated rats. However, administration of CGA for 10 weeks improved the GFR in diabetic rats significantly, implicating its nephroprotective action. We suggest that CGA improved GFR by downregulating TGF-β induced expression of extracellular matrix proteins in the glomerular matrix.

---

**Table:**

<table>
<thead>
<tr>
<th>Group</th>
<th>TGF-β Protein Levels</th>
<th>β-Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>DC</td>
<td>1.5 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>CGA-10</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>CGA-20</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

Fig. 5: Effect of CGA on TGF-β in kidney of STZ-induced diabetic rats.

NC: Normal control; DC: diabetic control; CGA-10: diabetic rats treated with CGA (10 mg/kg BW); CGA-20: diabetic rats treated with CGA (20 mg/kg BW).

Data are represented as mean ± S.D.

* P < 0.001 compared with normal control; ** P < 0.001 compared with diabetic control.
Proteinuria is an important indication of diabetic nephropathy which occurs as a result of decreased tubular reabsorption of plasma proteins [34, 35]. Our results showed that the total protein excreted in urine was significantly elevated in diabetic control rats. On the other hand, the total proteins excreted in urine were significantly decreased in CGA treated diabetic rats as compared to diabetic control rats. We suggest that CGA improved proteinuria by preventing hyperglycemia and TGF-β induced tubular injury that can induce glomerular hyper-filtration leading to protein infusion into Bowman's space.

Lipid peroxidation is an oxidative stress induced process leading to destruction and deterioration of unsaturated fatty acids present in plasma membrane. Lipid peroxidation of nephrons has been considered as one of the major risk factor for the development of nephropathy in type 2 DM [56]. It has been reported that the concentration of lipid peroxides increase in the kidney of diabetic rats and an increased level of TBARS has been considered as the index of lipid peroxidation [37]. From our results, it has been observed that in the diabetic control group, the level of TBARS was high due to lipid peroxidation as compared to normal control group. In chlorogenic acid treated diabetic rats, TBARS level was low in kidney, which may be due to the free radical scavenging activity of chlorogenic acid.

SOD is an enzyme present in cells which catalyzes the removal of superoxide radical (O2•−) and thus prevents the cell membrane and biological macromolecules from damage. Catalase is an antioxidant enzyme which catalyzes the detoxification of hydrogen peroxide [38]. SOD and catalase are the two most important enzymes of antioxidant machinery of cells. Reduced activities of SOD and catalase can have deleterious effects on kidney as a result of superoxide anion and hydrogen peroxide accumulation and damage. In the present study, activity of both these enzymes was reduced in diabetic untreated rats. However, the diabetic rats treated with CGA had a higher activity of SOD and catalase as compared to diabetic untreated rats, which further indicates that the CGA possesses significant antioxidant activity.

Histopathology of kidney showed similar findings. Different grades of pathological lesions were observed in the kidney tissue of diabetic rats including atrophy of tubular epithelium and mesangial expansion. The observed glomerular and tubular injury in diabetic untreated rats could be attributed to TGF-β stimulating expression of extracellular matrix proteins in glomerular mesangial cells, glomerular epithelial cells and tubular epithelial cells [39]. CGA treatment reversed almost all the abnormalities in nephrons, indicating effective protection against diabetic nephropathy.

Transforming growth factor (TGF-β) plays a pathogenic role in DN. TGF-β is up-regulated in renal fibrosis and its inhibition resulted in a potent reduction of fibrosis in DN [40]. The expression level of TGF-β was significantly down regulated in CGA supplemented groups compared to diabetic control group. Several studies have shown that high glucose concentrations stimulate the expression of TGF-β in diabetic rat kidney [41]. ROS like hydrogen peroxide is also reported to increase TGF-β and fibronectin production in mesangial cells [42]. All these findings confirm that hyperglycemia and ROS act together to stimulate the expression of TGF-β in kidney. Hence it is plausible to speculate that CGA down regulated the expression of TGF-β owing to its hypoglycaemic and antioxidant activities.

CONCLUSION

In the present investigation, oral administration of CGA to HFD/STZ induced Type-2 diabetic rat’s demonstrated prominent normalization of blood sugar level, HbA1c level, BUN, creatinine clearance, proteinuria and antioxidant status of kidney. Therefore, it can be concluded that the CGA possessed remarkably effective antidiabetic and nephroprotective potential against HFD/STZ induced Type-2 DM in Wistar rats. The findings of the present study is encouraging enough to warrant further studies on this phenolic compound in pursuit of a new effective ant diabetic and nephroprotective agent.

ACKNOWLEDGMENTS

This research work was not supported by any of the funding agency. We would like to acknowledge two anonymous reviewers who helped in shaping this research paper.

REFERENCES

16. McCarty MF. A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. Medical Hypotheses 2005; 64: 848-853.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the


