Renoprotective Effect of *Coccinia indica* Fruits and Leaves in Experimentally Induced Diabetic Rats

Mallur Somasundra Abignan Gurukar, Siddaiah Mahadevamma,
and Nandini Dattatreya Chilkunda

Department of Biochemistry and Nutrition, Council of Scientific and Industrial Research,
Central Food Technological Research Institute, Mysore, India.

ABSTRACT Diabetic nephropathy is one of the secondary complications of diabetes mellitus that is marked by changes in extracellular matrix components leading to end-stage renal failure. Diet plays an important role in managing diabetes. In the present study, the effect of *Coccinia indica* consumption on diabetes-mediated kidney damage was determined. Both control and diabetic rats were fed with AIN-76 diet supplemented with *C. indica* fruits and leaves individually at 10% and 5%, respectively, for a period of 2 months. Various parameters, such as fasting blood glucose, urine sugar, albumin excretion, kidney index, and glomerular filtration rate, were ameliorated to various extents by the supplementation of *C. indica* in the diet. Additionally, diabetic rats fed with diet supplemented with *C. indica* fruits or leaves showed improvement in glucose tolerance compared to control diabetic rats. They also exhibited beneficial effects on key antioxidant enzymes of the kidney. Furthermore, an increase in laminin and fibronectin as a result of diabetes was alleviated in *C. indica*-fed rats. These results indicate that the consumption of *C. indica* is beneficial in partially containing diabetes-mediated deleterious effects on the kidney.

KEY WORDS: • albumin excretion • *Coccinia indica* • diabetes mellitus • extracellular matrix components • kidney index

INTRODUCTION

DIABETES MELLITUS is one of the major causes of morbidity and mortality worldwide.¹ It is marked by sustained hyperglycemia, which in the long run leads to secondary complications such as diabetic nephropathy afflicting the kidney. Diabetic nephropathy is characterized by renal enlargement accompanied by marked changes in extracellular matrix (ECM) components.² Among the various ECM components, a decrease in heparan sulfate³ and chondroitin sulfate/dermatan sulfate⁴ and an increase in laminin, fibronectin, and type IV collagen⁵ have been observed. Remodeling of ECM components of the kidney in diabetic conditions is known to affect the filtration process.

There are various causes of development of diabetic nephropathy. Accumulating evidence suggests that free radicals, such as reactive oxygen species (ROS) and formation of advanced glycation end products, lead to the activation of various signaling factors, thereby bringing about changes in ECM components in the kidney.⁶

Among various strategies available to counter the deleterious consequences of diabetes, the diet plays a decisive role. Studies from our laboratory have shown that dietary components are efficient in containing diabetes-mediated complications, thereby helping maintain general health. Various dietary components, such as wheat bran and guar gum, butyric acid, the product obtained by anaerobic digestion of dietary fiber, fenugreek, and spent turmeric, have been shown to ameliorate the diabetic condition to various extents, imparting beneficial effects on the kidney.^{7–9}

Coccinia indica (synonym *C. cordifolia*) belongs to the Cucurbitaceae family and grows abundantly in India. It is a climbing perennial herb, growing throughout India especially in warmer and humid climatic conditions. It is widely used in traditional treatment of diabetes.¹⁰ The fruits are used for culinary purposes as a vegetable. Scientific investigations have supported the efficacy of leaf extracts in amelioration of diabetic conditions.¹¹ The juice of the roots and leaves is used to treat diabetes, and the aqueous and ethanolic extracts of the plant exhibit hypoglycemic action.¹² *C. indica* leaves have been shown to stimulate insulin secretion in diabetic rats.¹³ The present study evaluated the effects of feeding *C. indica* leaves and fruits on diabetic kidney disease, which has not been thus far reported despite its traditional use in diabetes.

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Address correspondence to: Nandini Dattatreya Chilkunda, MSc, PhD, Department of Biochemistry and Nutrition, Council of Scientific and Industrial Research-Central Food Technological Research Institute, JLB Road, Mysore 570 020, India, E-mail: cdnandini@yahoo.com

MATERIALS AND METHODS

Reagents

Streptozotocin (STZ), tert butyl hydroperoxide (TBHP), NADPH, hydrogen peroxide and glutathione reduced, paranitrophenylphosphate (pNPP), and CDNB were obtained from Sigma Chemicals Co. Antibodies to laminin and fibronectin, and secondary antibodies conjugated to ALP were obtained from Abcam. Glucose oxidase/oxidase (GOD/POD) and creatinine kits were purchased from Span Diagnostic Limited. Accu-Chek glucometer strips were obtained from Roche Diagnostics. All other chemicals used were of analytical grade.

Plant materials

C. indica fruits and leaves were collected from a local market during the month of August 2011 and were identified by a taxonomist. They were washed in running tap water and drained. The fruits were sliced and dried at 60°C for 30 h in an oven. Leaves were manually separated, washed in running tap water, and dried in an oven at 60°C for 24 h. The dried plant materials were powdered and stored in airtight containers in a refrigerator until use.

Analytical methods

The dietary fiber content was analyzed by the enzymatic-gravimetric method.¹⁴ Total sugar and uronic acid¹⁵ was estimated after acid hydrolysis of the materials. Gas-liquid chromatography (GC) analyses of the hydrolyzed samples were carried out by derivatizing the sugars.¹⁶

Isolation of phenols

C. indica fruits and leaves (2 g) were extracted with various solvents, such as acetone, methanol, ethanol, and water, on a magnetic stirrer for 2 h. The extracted mixture was centrifuged at 1200 g for 10 min. The supernatant was collected and filtered. The amount of phenolics was estimated as reported earlier.¹⁷

Animals

Male Wistar rats (OUTB-Wistar IND CFTRI) weighing around 105.0 ± 10.0 g were used for the study. The study was approved by the Institutional Animal Ethics Committee (IAEC-150/09). The animals were housed in individual cages with free access to food and water.

Preparation of diet

Diet (AIN-76) was freshly prepared. *C. indica* fruits and leaves were incorporated into the diet by replacing equal quantities of starch at 10% and 5% levels, respectively.

Grouping of animals and induction of diabetes mellitus

Rats were initially grouped into A and B based on the body weight. After 1 week of acclimatization, the rats in group B were injected with STZ at 47 mg/kg body weight in

a freshly prepared citrate buffer (0.1 M, pH 4.5),¹⁸ whereas the rats in group A were injected with the citrate buffer. After the injection, the rats were given 5% glucose water for 2 days to prevent drug-induced hypoglycemic shock. After 1 week of STZ injection, the diabetic status was confirmed by measuring the fasting blood glucose (FBG) levels. The animals were again subgrouped into respective control (FBG ≤ 100) and diabetic rats (FBG ≥ 120) based on the FBG levels. The groups were tentatively named as follows: starch-fed control/diabetic (SFC/SFD), *C. indica* fruit-fed control/diabetic (CFC/CFD), and *C. indica* leaf-fed control/diabetic (CLC/CLD). The control groups had 6 animals and the diabetic groups had 11 animals each at the start of the study. After 2 months of experimental period, there were seven, eight, and eight animals in the SFD, CFD, and CLD groups, respectively, and six animals each in the control groups.

Collection and analysis of various parameters in blood and urine

Blood was drawn from retro-orbital plexus into tubes containing heparin (20 U/mL blood). Blood glucose was determined by the GOD-POD method¹⁹ after prior fasting for 12 h. Urine of 24 h duration was collected over a layer of toluene by keeping them in metabolic cages. Reducing sugar in urine was measured by the dinitrosalicylic acid method.²⁰ Albumin in urine was measured by using the Albumin Blue 580 method.²¹ Creatinine was estimated in urine and serum by using a commercially available kit²² by which the glomerular filtration rate (GFR) was calculated.²³

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed after 45 days of feeding with *C. indica*-supplemented diet. Both control and diabetic rats were fasted overnight (for at least 12 h) and were given an oral glucose load of 2 g/kg body weight.^{24,25} Blood was drawn at 0 min (before giving glucose load) and at 20, 40, 80, and 120 min after glucose administration. The blood glucose level was measured using a glucometer. The area under the curve (AUC) was calculated using the trapezoidal rule.²⁶

Antioxidant enzymes activity in kidney

The measurement of antioxidant enzymes was carried out in kidney homogenates (0.1 g/mL) in saline. The homogenates were centrifuged at 31,000 g for 15 min, and an aliquot of supernatant was taken for the analyses. The catalase activity was assayed by following the rate of decomposition of hydrogen peroxide as described by Aebi.²⁷ The glutathione peroxidase activity was measured as described by Flohe and Gunzler.²⁸ The glutathione reductase activity was assayed by measuring the oxidation of NADPH by oxidized glutathione according to Carlberg and Mannervik.²⁹ The glutathione S-transferase activity was assayed using the CDNB and by measuring the CDNB-GSH complex formed as described by Warholm *et al.*³⁰

Relative quantitation of ECM components

ECM components were quantified by ELISA in kidney homogenates prepared by homogenizing tissue (0.1 g/mL) in lysis buffer (1% Triton X-100, 50 mM Tris, 150 mM EDTA, and protease inhibitor) and centrifuging at 2800 g for 15 min. Briefly, kidney homogenates (as 10 μ g protein) were coated overnight at 4°C. Nonspecific binding sites of antigen were blocked with 2% BSA. ECM components were detected by adding a specific antibody (1:3000 in 10 mM phosphate-buffered saline) directed against laminin and fibronectin and in individual wells overnight at 4°C. The secondary antibody conjugated with ALP was added (1:4000; 10 mM Tris buffer saline) and incubated at 37°C for 2 h. pNPP was used as substrate, and color developed was read at 405 nm in an ELISA plate reader. The fold changes in ECM components were determined by comparing nondiabetic control rats.

Statistical analyses

Data are expressed as mean $\pm$ SD, of $n=6$ in the control groups (SFC, CFC, and CLC) and $n=7$, 8, and 8 in the diabetic groups (SFD, CFD, and CLD, respectively). Statistical analyses of data were performed using one-way analysis of variance with a Tukey's multiple comparison post-test and with significance levels of $P<.05$, $P<.01$, and $P<.001$.

RESULTS

Determination of dietary fiber, total sugar, uronic acid, and analysis of neutral sugars by gas-liquid chromatography

C. indica fruits and leaves showed good amounts of both soluble and insoluble fibers and uronic acid-containing polysaccharides as given in Table 1. Fruits had slightly more soluble fiber than leaves. GC analysis of the whole fruit and leaf powder for neutral sugars showed the presence of arabinose, xylose, mannose, galactose, and glucose as major sugars in fruit, whereas leaves also contained rhamnose (data not shown).

Determination of phenolic content in *C. indica* fruits and leaves

Phenols were extracted in various hydrated solvents and quantitated. Both fruits and leaves contained polyphenols (Table 2). Attempts were also made to determine the types

of phenols. There were many peaks that could not be identified with the standards used by us (data not shown).

Effect of *C. indica* fruits and leaves on diet and water intake and gain in body weight

Experimentally induced diabetic rats were supplemented with *C. indica* fruits and leaves individually at 10% and 5% levels (w/w), respectively, in AIN-76 diet. Animals were monitored for feed and water intakes and gain in body weight once every 15 days for 2 months. Diabetic animals in both untreated and treated groups exhibited marked hyperphagia. Feed consumption was significantly higher in all the diabetic groups compared to their respective nondiabetic control groups. The presence of *C. indica* fruits and leaves did not affect the food intake indicating that there were no issues with palatability. Diabetic animals also exhibited polydipsia, which was prominent in all the groups. Although diabetic animals consumed more feed, there was a significant reduction in body weight compared to control animals. The increase in body weight was observed in the treated group when compared to the untreated rats among diabetic animals (Fig. 1).

Effect of *C. indica* fruits and leaves on fasting blood sugar, albumin excretion, GFR, and kidney index

There was a significant increase of more than two- to threefold in blood glucose levels in diabetic animals when compared to nondiabetic control animals. The increase was significantly ameliorated by 34% and 22% on feeding diet supplemented with *C. indica* fruits and leaves, respectively. Reducing sugar excreted in urine was higher in SFD when compared to SFC, but lower when *C. indica* fruits and leaves were added to the diet (Fig. 2A, B). Albumin excretion in the urine was significantly increased by fourfold in the untreated diabetic rats when compared to the control rats. *C. indica* in the diet prevented the increase resulting in the net increase of 2.5-fold when compared to nondiabetic control animals (Fig. 3C). Glomerular filtration and kidney index rate were higher in diabetic animals, but were lower to various extents in *C. indica*-fed animals (Fig. 3A, B).

Effect of *C. indica* fruits and leaves on OGTT

Both *C. indica*-treated and -untreated animals showed high blood glucose levels characteristic of diabetes after oral

TABLE 1. CARBOHYDRATE AND DIETARY FIBER CONTENT OF *COCCINIA INDICA* FRUITS AND LEAVES

	Fruit	Leaf
Total sugar (μ g/mg powder)	274 $\pm$ 19	225 $\pm$ 14
Uronic acid (μ g/mg powder)	56.5 $\pm$ 6.3	52.5 $\pm$ 4.9
Soluble dietary fiber (g/100 g powder)	10 $\pm$ 0.5	9.8 $\pm$ 0.25
Insoluble dietary fiber (g/100 g powder)	39.5 $\pm$ 1.5	46.2 $\pm$ 2.5

Values are given as mean $\pm$ SD of two independent determinations.

TABLE 2. TOTAL POLYPHENOL CONTENT OF *COCCINIA INDICA* FRUITS AND LEAVES EXTRACTED FROM DIFFERENT SOLVENTS

	Solvents			
	Acetone	Methanol	Ethanol	Aqueous
<i>Coccinia</i> fruit (μ g/mg powder)	9.5 $\pm$ 0.19	7.2 $\pm$ 0.06	8.0 $\pm$ 0.12	8.2 $\pm$ 0.12
<i>Coccinia</i> leaves (μ g/mg powder)	6.9 $\pm$ 0.3	6.4 $\pm$ 0.15	6.5 $\pm$ 0.30	3.8 $\pm$ 0.03

Values are given as mean $\pm$ SD of two independent determinations.

FIG. 1. Effect of *Coccinia* fruits and leaves on gain in body weight (A), diet intake (B), and water intake (C). Values are mean $\pm$ SEM of n rats in the control groups ($n=6$), SFD ($n=7$), CFD ($n=8$), and CLD ($n=8$). ^aSFC versus SFD; ^bSFD versus *C. indica*-treated diabetic groups with significant levels designated as $*P<.05$, $**P<.01$, $***P<.001$. SFC, starch-fed control; SFD, starch-fed diabetic; CFC, *C. indica* fruit-fed control; CFD, *C. indica* fruit-fed diabetic; CLC, *C. indica* leaf-fed control; CLD, *C. indica* leaf-fed diabetic.

FIG. 2. Effect of *Coccinia* fruits and leaves on fasting blood sugar (A) and urine sugar (B). Values are mean $\pm$ SEM of n rats in the control groups ($n=6$), SFD ($n=7$), CFD ($n=8$), and CLD ($n=8$). ^aSFC versus SFD; ^bSFD versus *C. indica*-treated diabetic groups with significant levels designated as $*P<.05$, $**P<.01$, $***P<.001$.

glucose load. Both *C. indica* fruits and leaves were able to prevent a rapid raise in blood glucose levels in diabetic animals as evidenced by AUC (Fig. 4). The decrease in AUC in CFD and CLD was 11% and 11.5%, respectively.

Effect of *C. indica* fruits and leaves on ECM components in kidney

Since the dietary materials tested exhibited beneficial effects on kidney index, microalbumin excretion, and GFR, their effect on quantitative changes of major glycoconjugates, laminin, and fibronectin of the ECM was determined. There was a significant increase in these major ECM components as a result of diabetes. The increase was prevented to various extents by feeding *C. indica* fruits and leaves (Fig. 5). There were no significant quantitative changes in nondiabetic controls irrespective of the presence or absence of dietary materials.

Effect of *C. indica* fruits and leaves on antioxidant enzymes in kidney

A diet supplemented with *C. indica* fruits and leaves is able to ameliorate the decreased activity of various antioxidant enzymes, such as catalase, glutathione peroxidase,

FIG. 3. Effect of *Coccinia* fruits and leaves on kidney index (A), glomerular filtration rate (GFR) (B), and microalbuminuria (C). Values are mean $\pm$ SEM of n rats in the control groups ($n=6$), SFD ($n=7$), CFD ($n=8$), and CLD ($n=8$). ^aSFC versus SFD; ^bSFD versus *C. indica*-treated diabetic groups with significant levels designated as * $P < .05$, ** $P < .01$, *** $P < .001$.

glutathione reductase, and glutathione S-transferase, during diabetes, which helps to a large extent in combating oxidative stress (Fig. 6).

DISCUSSION

The present study deals with the effects of *C. indica* fruit and leaf consumption on kidneys of experimentally induced

FIG. 4. Effect of *Coccinia* fruits and leaves on the glucose tolerance test (A) and area under the curve (AUC) (B). Values are mean $\pm$ SEM of n rats in the control groups ($n=6$), SFD ($n=7$), CFD ($n=8$), and CLD ($n=8$). ^aSFC versus SFD; ^bSFD versus *C. indica*-treated diabetic groups with significant levels designated as * $P < .05$, ** $P < .01$, *** $P < .001$.

diabetic animals. *C. indica* fruits are commonly consumed as vegetable in India. Both *C. indica* fruits and leaves are rich in fiber and bioactive compounds. Earlier, *C. indica* leaves were shown to be antihyperglycemic.¹⁰ This study was evaluated whether their consumption will ameliorate symptoms of diabetic nephropathy. Hence, experiments were carried out by feeding experimentally induced diabetic rats with the diet supplemented with *C. indica* fruits and leaves. The increase in feed intake, water intake, and changes in gain in body weight was significantly ameliorated with the supplementation. Significant increases of more than two- to threefold in blood glucose and reducing sugar in urine were ameliorated to various extents on feeding a diet supplemented with *C. indica* fruits and leaves, respectively.

Kidney damage is one of the hallmarks of diabetic nephropathy. Sustained hyperglycemia leads to secondary complications, such as diabetic nephropathy, which eventually leads to end-stage renal disease.¹³ Increased renal enlargement, albumin excretion, and GFR are the prominent features. One of the causative factors reported is the increased production of ROS. Available evidence suggests

FIG. 5. Effect of *Coccinia* fruits and leaves on laminin (A) and fibronectin (B). Values are mean $\pm$ SEM of n rats in the control groups ($n=6$), SFD ($n=7$), CFD ($n=8$), and CLD ($n=8$). ^aSFC versus SFD; ^bSFD versus *C. indica*-treated diabetic groups with significant levels designated as * $P < .05$, ** $P < .01$, *** $P < .001$.

that oxidative stress in the kidney increases as a result of increased uptake of glucose, which then leads to increased ROS production.³¹ The parameters relating to diabetic nephropathy were prevented to various extents in *C. indica*-fed animals indicating beneficial effects. Our studies show that feeding *C. indica* fruits and leaves is able to ameliorate the decreased activity of various antioxidant enzymes, such as catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase, during diabetes, which helps to a large extent in combating oxidative stress that is consistent with the other literature reports.¹¹

Increased ROS leads to dysregulation of various molecules, namely, growth factor production, resulting in the increased production of ECM components.³² Renal enlargement is one of the earliest events of diabetic nephropathy. Thus, kidney index increased in diabetic condition. ECM components, such as laminin and fibronectin, were found to increase in diabetic rats, which are in accordance with the literature reports.⁵ The increase in ECM components leads to glomerular basement thickening and expansion of the mesangial matrix compromising the filtration process. *C. indica* in diet was able to significantly negate the increased synthesis.

The beneficial effects observed in the kidney by feeding *C. indica* could be a result of controlling blood glucose

FIG. 6. Effect of *Coccinia* fruits and leaves on catalase (A), glutathione reductase (B), glutathione peroxidase (C), and glutathione S-transferase (D). Values are mean $\pm$ SEM of n rats in the control groups ($n=6$), SFD ($n=7$), CFD ($n=8$), and CLD ($n=8$). ^aSFC versus SFD; ^bSFD versus *C. indica*-treated diabetic groups with significant levels designated as * $P < .05$, ** $P < .01$, *** $P < .001$.

through mediators, such as phytochemicals and/or dietary fiber, and through secondary metabolites, such as short-chain fatty acids. In our experiment, whole leaves and fruits were used rather than selective fractions to simulate commonly consumed conditions and obtain the full range of bioactive compounds. Antihyperglycemic properties of *C. indica* fruits and leaves in diabetic animals are well documented.³³ *C. indica* extract was earlier shown to increase insulin levels in blood hinting at β -cell stimulating activity.³⁴ In a similar way, flavonoids and glycosides present in *C. indica* leaves are reported to have antidiabetic effects.³⁵ Triterpenes present in the toluene extract of *C. indica* were shown to reduce alloxan-induced β -cell damage and increase insulin secretion. Oral administration of pectin isolated from *C. indica* fruits was shown to have a significant hypoglycemic effect in normal rats. Pectin is a soluble fiber that affects blood glucose levels either by decreasing the transit time or indirectly through the production of short-chain fatty acids. Acetate, propionate, and butyrate are the commonly produced SCFAs obtained by anaerobic fermentation of dietary fiber components by the microflora in the large intestine. Butyrate, in particular, is known to act at the level of gene expression^{36,37} and recently was demonstrated to affect insulin sensitivity.³⁷ Furthermore, butyrate was shown to act against oxidative stress-induced DNA damage,³⁸ which may exert beneficial effects in the diabetic condition.

CONCLUSION

C. indica fruits and leaves are rich in fiber and bioactive compounds. Their consumption is beneficial on diabetes-mediated kidney damage either by directly decreasing blood glucose or through the action of various compounds that decrease oxidative stress.

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REFERENCES

1. Zimmet P, Alberti KGMM, Shaw J: Global and societal implications of the diabetes epidemic. *Nature* 2000;1414:782–787.
2. Roger M, Wahab NA: Extracellular matrix metabolism in diabetic nephropathy. *J Am Soc Nephrol* 2001;14:1358–1373.
3. Reddi AS, Glomerular and urinary glycosaminoglycans in diabetic rats: *Clin Chim Acta* 1990;189:211–220.
4. Darukeshwara J, Salimath PV, Nandini CD: Diabetes results in structural alteration of chondroitin sulphate/dermatan sulphate in the rat kidney: effects on the binding to extracellular matrix components. *Glycobiology* 2011;21:960–972.
5. Ayo SH, Radnik RA, Glass WF, Garoni JA, Rampt ER, Appling DR, Kreisberg JI: Increased extracellular matrix synthesis and mRNA in mesangial cells grown in high-glucose medium. *Am J Pathol* 1990;36:1339–1348.
6. Bonke VT, Lindschau C, Rizkalla B, Bach LA, Boner G: Attenuation of extracellular matrix accumulation in diabetic nephropathy by the advanced glycation end product cross-links breaker rat-711 via a protein kinase c-dependent pathway. *Diabetes* 2004;53:2921–2930.
7. Chethan Kumar M, Rachappaji KS, Nandini CD, Sambaiah K, Salimath PV: Modulatory effect of butyric acid a product of dietary fiber fermentation in experimentally induced diabetic rats. *J Nutr Biochem* 2002;13:522–527.
8. Puneeth Kumar A, Mallikarjun C, Nandini CD, Salimath PV: Effect of butyric acid supplementation on serum and renal anti-oxidant enzyme activities in streptozotocin-induced diabetic rats. *J Food Biochem* 2010;34:15–30.
9. Shetty AK, Suresh Kumar G, Sambaiah K, Salimath PV: Effect of bitter gourd (*Momordia charantia*) on glycaemic status in Streptozotocin induced diabetic rats. *Plant Foods Hum Nutr* 2005;60:109–112.
10. Venkateswaran S, Pari L: Effect of *Coccinia indica* on blood glucose, insulin and key hepatic enzymes in experimental diabetes. *Pharmaceut Biol* 2002;3:165–170.
11. Venkateswaran S, Pari L: Effect of *Coccinia indica* leaf extract on plasma antioxidants in Streptozotocin-induced experimental diabetes in rats. *Phytother Res* 2003;17:605–608.
12. Akhtar MA, Rashid M, Wahed MI, Islam R: Comparison of long-term antihyperglycemic and hypolipidemic effects between *Coccinia cordifolia* (Linn.) and *Catharanthus roseus* (Linn.) in alloxan-induced diabetic rats. *Res J Med Med Sci* 2007;2:29–34.
13. Kumar GP, Sudheesh S, Vijayalakshmi NR: Hypoglycaemic effect of *Coccinia indica*: mechanism of action. *Planta Med* 1993;59:330–332.
14. Asp NG, Johansson CG, Hallmer H, Siljestroem M: Rapid enzymic assay of insoluble and soluble dietary fiber. *J Agric Food Chem* 1983;31:476–482.
15. Dische Z, A new specific color reaction of hexuronic acid: *J Biol Chem* 1947;167:189–198.
16. Sawardekar JS, Slonekar LS, Jeanes A: Quantative determination of monosaccharide's as their alditole acetates by gas liquid chromatography. *Anal Chem* 1967;39:430–435.
17. Nordkvist E, Salomonsson AC, Aman P: Distribution of insoluble bound phenolic acids in barley grain. *J Sci Food Agric* 1984;35:657–661.
18. Hatch GM, Cao SG, Angl A: Decreases in cardiac phosphatidyl glycerol in Streptozotocin induced diabetic rats do not affect cardiolipin biosynthesis: evidence for distant pools of phosphatidyl glycerol in the heart. *Biochem J* 1995;306:759–764.
19. Hugget ASG, Nixon DA: Use of glucose oxidase, peroxidase and O-dianisidine in the determination of blood glucose and urinary glucose. *Lancet* 1957;270:368–370.
20. Miller GL: Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 1959;31:426.
21. Kessler MA, Meinitzer A, Petek W, Wolfbesis OS: Micro-albuminuria and borderline-increased albumin excretion determined with a centrifugal analyzer and the Albumin Blue 580 fluorescence assay. *Clin Chem* 1997;43:996–1002.
22. Bowers LD: Kinetic serum creatinine assays. The role of various factors in determining specificity. *Clin Chem* 1980;26:551–554.
23. Yokozawa T, Chung HY, Qun HE, Oura H: Effectiveness of green tea tannin on rats with chronic renal failure. *Biosci Biotech Biochem* 1996;60:1000–1005.

24. Du Vigneaud V, Karr WG: Carbohydrates utilization rate of disappearance of D-glucose from the blood. *J Biol Chem* 1996; 66:281–300.
25. Al-Awadi FW, Khattar MA, Gumaa A: The mechanism of the hypoglycemic effect of a plant extract. *Diabetologia* 1985;28: 432–434.
26. Dragomir SS, Cerone P, Sofo A: Some remarks on the trapezoid rule in numerical integration. *Indian J Pure Appl Math* 2000;31: 475–494.
27. Aebi H: Catalase *in vitro*. *Methods Enzymol* 1984;105:121–126.
28. Flohe L, Gunzler WA: Assays of glutathione peroxidase. *Methods Enzymol* 1984;105:114–121.
29. Carlberg I, Mannervik B: Glutathione reductase. *Methods Enzymol* 1984;113:484–490.
30. Warholm M, Guthenberg C, Bahr CV, Mannervik B: Glutathione transferase from human liver. *Methods Enzymol* 1985;113:499–504.
31. Forbes JM, Coughlan MT, Coope ME: Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 2008;57: 1446–1454.
32. Mason RM, Wahab NA: Extracellular matrix metabolism in diabetic nephropathy. *Am Soc Nephrol* 2003;14:1358–1373.
33. Sutradhar BK, Islam J, Shoyeb A, Khaleque HN, Sintaha M: An evaluation of antihyperglycemic and antinociceptive effects of crude methanol extract of *Coccinia grandis* (L.) J. Voigt. (Cucurbitaceae) leaves in Swiss Albino mice. *Adv Nat Appl Sci* 2011;5:1–5.
34. Kuriyan R, Rajendran R, Bantwal G, Kurpad AV: Effect of supplementation of *Coccinia cordifolia* extract on newly detected diabetic patients. *Diabetes Care* 2008;31:216–220.
35. Ajay SS: Hypoglycemic activity of *Coccinia indica* (Cucurbitaceae) leaves. *Int J Pharm Tech Res* 2009;1:892–893.
36. Smith JG, Yokoyama WH, German JB: Butyric acid from the diet: actions at the level of gene expression. *Crit Rev Food Sci* 1998;38:259–297.
37. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Jianping Y: Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009;58:1509–1517.
38. Rosignoli R, Fabiani R, Bartolomeo AD, Spinozzi F, Agwa E, Pelli MA, Morozzi G: Protective activity of butyrate on hydrogen peroxide-induced DNA damage in isolated human colonocytes and HT29 tumour cells. *Carcinogenesis* 2001;22:1675–1680.