British Journal of Nutrition (2010), **104**, 1787–1795
doi:10.1017/S0007114510002977

Amelioration of hyperglycaemia and its associated complications by finger millet (*Eleusine coracana* L.) seed coat matter in streptozotocin-induced diabetic rats

Shanmugam Shobana[1], Mysore R. Harsha[1], Kalpana Platel[2], Krishnapura Srinivasan[2]* and Nagappa G. Malleshi[1]

[1]*Department of Grain Science and Technology, Central Food Technological Research Institute, CSIR, Mysore 570 020, India*
[2]*Department of Biochemistry and Nutrition, Central Food Technological Research Institute, CSIR, Mysore 570 020, India*

(Received 17 December 2009 – Revised 25 March 2010 – Accepted 5 May 2010 – First published online 28 October 2010)

Finger millet (*Eleusine coracana*) is extensively cultivated and consumed in India and Africa. The millet seed coat is a rich source of dietary fibre and phenolic compounds. The effect of feeding a diet containing 20 % finger millet seed coat matter (SCM) was examined in streptozotocin-induced diabetic rats. Diabetic rats maintained on the millet SCM diet (diabetic experimental (DE) group) for 6 weeks exhibited a lesser degree of fasting hyperglycaemia and partial reversal of abnormalities in serum albumin, urea and creatinine compared with the diabetic control (DC) group. The DE group of rats excreted comparatively lesser amounts of glucose, protein, urea and creatinine and was accompanied by improved body weights compared with their corresponding controls. Hypercholesterolaemia and hypertriacylglycerolaemia associated with diabetes were also notably reversed in the DE group. Slit lamp examination of the eye lens revealed an immature subcapsular cataract with mild lenticular opacity in the DE group of rats compared to the mature cataract with significant lenticular opacity and corneal vascularisation in the DC group. Lower activity of lens aldose reductase, serum advanced glycation end products and blood glycosylated Hb levels were observed in the DE group. The millet SCM feeding showed pronounced ameliorating effects on kidney pathology as reflected by near normal glomerular and tubular structures and lower glomerular filtration rate compared with the shrunken glomerulus, tubular vacuolations in the DC group. Thus, the present animal study evidenced the hypoglycaemic, hypocholesterolaemic, nephroprotective and anti-cataractogenic properties of finger millet SCM, suggesting its utility as a functional ingredient in diets for diabetics.

Finger millet: Seed coat matter: Antidiabetic effects: Hypocholesterolaemic: Nephroprotective: Anti-cataractogenic effects

Diabetes mellitus is a metabolic disorder characterised by hyperglycaemia resulting from a defect in insulin action or deficiency in insulin secretion or both leading to alterations in carbohydrate, lipid and protein metabolism. Uncontrolled diabetes leads to several microvascular (neuropathy, nephropathy and retinopathy) and macrovascular (atheroma) complications that affect many organs of the body. The incidence of diabetes is alarmingly high worldwide (2·8 %) and afflicted about 66·6 million people in India in 2004[(1)].

A multitude of herbs, spices and other plant materials have been in use for the treatment of diabetes[(2)]. Diet plays an important role in the management of diabetes mellitus. In this context, the health-beneficial effects of dietary fibre and antioxidants derived from plant food sources have been extensively studied. Whole grain cereals form the most important sources of dietary fibre, minerals and phytochemicals with antioxidant activity. Consumption of whole grains has been prospectively associated with lower risk of diabetes[(3)] and CVD[(4)]. The constituents of whole grains that contribute to the reduced risk of diabetes include complex carbohydrates, dietary fibre and phytochemicals such as polyphenols and phytates. In most cereals, the phytochemical constituents possessing health beneficial attributes are largely concentrated in the seed coat and several methods have been developed to prepare the phytochemical-rich fraction or to isolate the seed coat components.

Traditionally, finger millet food preparations are known for their higher sustaining power, lower glycaemic response and higher satiety scores compared with other cereal foods and are usually recommended for diabetics. The effect of feeding finger millet and kodo millet on the antioxidant and glycaemic status of alloxan-induced diabetic rats has been reported, which evidenced a 36 and 42 % reduction in the blood glucose levels and 13 and 27 % reduction in cholesterol levels, respectively, besides improving the antioxidant status[(5)]. Finger millet seed coat is a reserve of several phenolic compounds, such as phenolic acids, flavonoids, polymeric tannins and anthocyanins, and is an effective inhibitor of pancreatic

Abbreviations: AGE, advanced glycation end product; AR, aldose reductase; DC, diabetic control; DE, diabetic experimental; GFR, glomerular filtration rate; NC, normal control; NE, normal experimental; SCM, seed coat matter.

* **Corresponding author:** Dr K. Srinivasan, fax +91 821 2517233, email ksri.cftri@gmail.com

amylase and intestinal α-glucosidase[6,7]. Finger millet seed coat is also a rich source of phytate and minerals[8]. Dietary polyphenols and phytates are known for their ability to reduce carbohydrate digestibility and thereby regulate post-prandial glycaemic response[9]. Moreover, polyphenols are known to inhibit glucose absorption and also prevent advanced glycation end product (AGE) formation[10]. However, reports on the ameliorative effects of the millet seed coat on the metabolic abnormalities and complications associated with diabetes are scanty. In view of this, the influence of finger millet seed coat matter (SCM) on diabetic consequences in streptozotocin-induced diabetic rats was studied.

Experimental methods

Chemicals

Streptozotocin, glucose oxidase, *o*-dianisidine, horseradish peroxidase, Triton X-100, human serum albumin, Trizma base, thiobarbituric acid, 1,1,3,3-tetramethoxypropane and bile salts were purchased from Sigma Chemical Company (St Louis, MO, USA). Thiosemicarbazide, diacetyl monoxime, cholesterol, Bernhardt–Tommarelli-modified salt mixture and sodium azide were purchased from SISCO Research Laboratories (Mumbai, India). Succinic acid was purchased from SD Fine Chemicals (Mumbai, India). Casein was purchased from Nimesh Corporation (Mumbai, India). Maize starch, cane sugar powder and refined peanut oil were purchased from the local market. All other chemicals and solvents used were of analytical grade from Qualigens Fine Chemicals (Mumbai, Maharashtra, India). The solvents were distilled before use.

Finger millet seed coat matter

Finger millet (GPU 28 variety) procured from the University of Agricultural Sciences, Bangalore, India, was cleaned before use. SCM was prepared from finger millet according to the protocol described previously[7]. The millet was moistened by spraying with 7 % (w/v) water, equilibrated for 10 min and pulverised in a carborundum disc mill. The meal was sifted through an 85-mesh stainless steel sieve (180 μm openings) and the tailings were again pulverised and sifted through the same sieve. The process of pulverising the tailings and sieving were repeated two more times. The millet endosperm flour fraction that passed through the first, second and third stages of sieving was pooled and termed as refined finger millet flour. The unsievable tailings (which are >85-mesh size) at the end of the third pulverising were collected and termed as millet SCM.

Experimental animals

Male Wistar rats (weighing 150–160 g) raised at the experimental animal production unit of our institute were used in the present study. The animal study was conducted taking all precautions to minimise the pain or discomfort to the animals and with due approval from the institutional animal ethics committee. Diabetes was induced by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight as 1 ml freshly prepared solution in 0·1 M citrate buffer, pH 4·5). The rats were provided 5 % glucose solution in place of water for the first 48 h to prevent the initial drug-induced hypoglycaemic mortality. At 3 d, after the injection, blood was drawn from the retro-orbital plexus of the overnight-fasted rats and was used to assess their hyperglycaemic status by the estimation of blood glucose levels. The rats with the fasting blood glucose levels higher than 2500 mg/l were considered as diabetic.

The rats were divided into four groups out of which two groups were diabetic (twelve rats in each group) and the other two groups were non-diabetic (eight rats in each group). The rats were housed in individual stainless-steel metabolism cages with free access to diet and water. The feeding trial was for a duration of 6 weeks. One of the diabetic groups received the millet SCM-containing diet (diabetic experimental (DE)) and the other received the basal control diet (diabetic control (DC)). Similarly, one of the non-diabetic groups received the millet SCM-containing diet (normal experimental (NE)) and the other received basal control diet (normal control (NC)). The basal control diet contained 54 % maize starch, 21 % casein, 10 % cane sugar, 10 % refined peanut oil, 4 % Bernhardt–Tommarelli modified mineral mixture and 1 % National Research Council vitamin mixture. In the case of the experimental diet, 20 % millet SCM was incorporated at the expense of an equivalent amount of maize starch.

The 24 h urine samples were collected at weekly intervals for the determination of urinary metabolites. The urine samples were filtered, the volume was noted and stored frozen until further analysis. At the end of the experimental period, to observe cataract formation, ophthalmological examination was conducted using slit lamp detectors. The rats were killed under mild diethyl ether anaesthesia by exsanguination from the heart. The blood samples were collected by puncturing the heart and the serum was separated by centrifugation and stored at −20°C until further analysis. A portion of the blood was also collected in tubes containing EDTA as anticoagulant and was immediately utilised for the analysis of HbA1c levels. The eye lens and kidneys were excised and used for the determination of aldose reductase (AR) activity and for histological studies, respectively.

Analytical methods

Proximate composition of the SCM was determined by the approved American Association of Cereal Chemists methods[11]. Total dietary fibre was determined by the rapid enzymic assay according to the method of Asp *et al.*[12]. The analytical values were expressed as the mean of three independent determinations for each sample.

Extraction and estimation of phenolics. The SCM (1 g) was refluxed in 100 ml of 1 % HCl in methanol at 60°C for 10 min and the residue was re-extracted with fresh solvent three more times. The extracts were filtered through Whatman no. 1 filter paper and the filtrates were pooled together and concentrated to 2–3 ml under vacuum in a rotary flash evaporator and was used for the assay of total polyphenols[6]. Total phenolic content of the millet seed coat extract (1 % HCl-methanolic extract) was assayed according to the method of Singleton *et al.*[13].

Plasma and urinary glucose were measured by the glucose oxidase method of Huggett & Nixon[14]. Serum and urinary

urea levels were estimated by the method of Wybenga *et al.*[15]. Creatinine in serum and urine was estimated by Folin's method as described by Oser[16] by adding the samples with alkaline picrate solution. Total protein levels in urine and serum were estimated according to the method of Bradford[17]. Serum albumin was quantified according to the dye-binding method of Cooper[18].

Serum cholesterol was estimated by the $FeSO_4$–H_2SO_4 method of Searcy & Bergquist[19]. Serum HDL-cholesterol was determined in the supernatant after precipitation of the apo-B-containing lipoproteins with heparin-Mn^{++} according to the procedure of Warnick & Albers[20]. The precipitate was extracted with chloroform–methanol (2:1, v/v) solvent mixture and an aliquot of the extract was used for the determination of LDL + VLDL cholesterol. Serum TAG were determined according to the method of Fletcher[21].

Aldose reductase activity in the eye lens was determined according to the method of Kim & Oh[22]. Eye lenses were homogenised with potassium phosphate buffer (0·135 M, pH 7·0) containing 0·5 mM-phenyl methyl sulphonyl fluoride and 10 mM-β-mercaptoethanol. The homogenate was centrifuged at 10 000 ***g*** for 30 min at 4°C. The supernatant was used for the enzyme activity measurement. Serum AGE were determined by the method of Monnier & Cerami[23]. Serum samples were appropriately diluted with 0·05 M-sodium phosphate buffer (pH 7·4) to adjust the protein concentration to about 10 μg/ml and were used for fluorescence measurement. AGE-related fluorescence was measured from the emission fluorescence spectra (400–500 nm) of diluted serum samples with excitation at 370 nm in a spectrofluorometer. Glycosylated Hb (HbA1c) levels were estimated by using the Presice kit (Nycocard Axisheild, Oslo, Norway) and the values were expressed as % of Hb.

Glomerular filtration rate (GFR) was calculated using the formula:

$$\frac{\text{Urinary creatinine (mg/l)} \times \text{urine volume (ml)} \times 1000\,\text{g}}{\text{Serum creatinine (mg/l)} \times \text{body weight (g)} \times 1440\,\text{(min)}} = \text{GFR (ml/min)}.$$

Atherogenic index was calculated according to the formula[24]:

$$\text{Atherogenic index} = \frac{\text{Total cholesterol} - \text{HDL-cholesterol}}{\text{HDL-cholesterol}}.$$

Opthalmological examination

Eye lenses of the experimental rats were examined for cataract-associated changes at the end of the experimental period using a slit lamp equipped with a built-in camera (Carl Zeiss Meditech AG, 07 740 Jena, Germany).

Histopathological studies

Kidneys were immediately fixed in 10 % formalin and were dehydrated and embedded in paraffin wax for sectioning (5 (SEM 1) μm thick) in a microtome. Kidney sections were stained with haematoxylin and eosin and were examined under a light microscope for changes in the glomerulus and the tubules.

Statistical analysis

Values are expressed as means with their standard errors of eight rats. Statistical analysis was carried out using Prism Graphpad Instat statistical software (GraphPad Software, Inc., La Jolla, CA, USA). Results were analysed and the significance level was calculated using the Tukey–Kramer multiple comparison test; comparisons between groups were made by means of one-way ANOVA. The differences were considered significant at $P<0·05$.

Results

The SCM of finger millet contained 11·2 % polyphenols, 13·6 % protein, 3·2 % fat, 19 % starch and 48 % dietary fibre. The experimental diet containing 20 % of SCM provides 2·24 % of polyphenols, which is nearly the same as that of the polyphenol content of the millet whole meal (2·3 g%). The experimental diet also contained about 9·6 % of dietary fibre.

Countering of weight loss

All the diabetic rats fed with the experimental diet (DE group) survived the experimental period of 6 weeks, whereas nine out of twelve diabetic rats fed with the control diet (DC group) survived the course of the experimental period. The rats consumed the control as well as the experimental diets normally and dietary intake was comparatively higher in the diabetic groups compared with the non-diabetic groups. However, no significant differences were observed with respect to dietary intake between the DC and DE groups and also between the NC and NE groups. The body weights of the diabetic rats remained much lower than those of corresponding non-diabetic rats (DC). While a significant weight loss was observed in the DC group of rats ($P<0·001$), those diabetic rats maintained on the experimental diet showed a significant improvement in body weight throughout the experimental duration as compared to the rats fed with the control diet ($P<0·001$) (Fig. 1(a)). There was no difference in body weight among the non-diabetic groups (NC and NE) of rats.

Improved blood parameters

The mean fasting blood glucose concentration of the DC group of rats which was 3430 mg/l at the beginning of the study did not change appreciably until the end (Fig. 1(b)), whereas the mean fasting blood glucose of the DE group of rats decreased by 31 % (from the initial 3130 to 2160 mg/l at the end of the experimental period). The mean fasting blood glucose value of DE group of rats at the end of 6 weeks was nearly 39 % lower than that of the DC group of rats ($P<0·001$). This clearly indicated that feeding the diabetic animals with a diet containing the millet SCM helps in the regulation of hyperglycaemia.

Diabetic condition resulted in a decrease in total serum protein, particularly albumin, and an increase in urea and creatinine levels (Table 1). The DE group of rats exhibited improved total protein and albumin levels in addition to

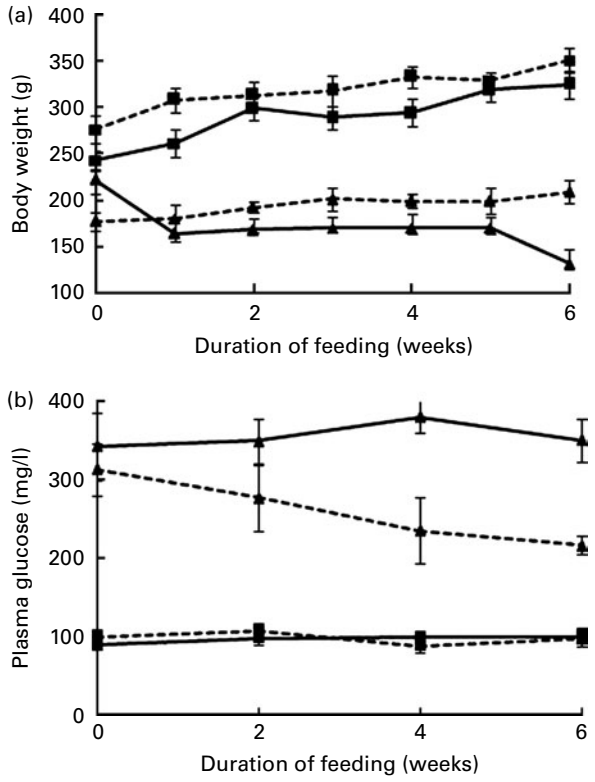

Fig. 1. Influence of dietary finger millet seed coat matter on (a) body weight and (b) blood glucose levels in diabetic rats. NC, normal control diet (–■–); NE, normal experimental diet (- -■- -); DC, diabetic control diet (–▲–); DE, diabetic experimental diet (- -▲- -).

lowered levels of urea and creatinine in the serum, compared with those of the DC group. Serum urea (490 mg/l) and creatinine (8·9 mg/l) levels were lower in the DE group (by 13 and 36 %, respectively) compared to their levels in the DC group (570 and 14·0 mg/l for urea and creatinine, respectively). The DE group of rats exhibited higher levels of serum total protein (57·0 mg/l, $P<0·01$), as well as albumin levels (28·0 mg/l, $P<0·05$) compared with the DC group (44·0 and 19·0 mg/l, respectively) which corresponded to an increase of 30 and 47 %, respectively, compared with the DC group. The albumin:globulin ratio in the DC group (0·76) was significantly lower than that of NC group. Diabetic rats maintained on the SCM diet (DE) showed an albumin:globulin ratio (0·97) which was almost comparable to those of the NC group. There were no significant differences among the two non-diabetic (NC and NE) groups of rats in the serum concentrations of total protein, albumin, urea and creatinine (Table 1).

Diabetic rats maintained on the SCM diet showed significantly lower serum cholesterol levels (as much as 43 %) as compared to those of the DC group of rats (Table 2). The reduction in cholesterol observed in the DE group was mainly from the LDL + VLDL-associated fraction of cholesterol (68 % lower compared to that of DC group). Although the increase in HDL-associated cholesterol fraction of the DE group was not significantly higher compared to that of the DC group, the same was 65 % higher ($P<0·001$) in normal rats as a result of finger millet SCM feeding. The atherogenic index is an indicator of the tendency to develop atherosclerosis, a profound diabetic complication. The ratio of LDL + VLDL-cholesterol to HDL-cholesterol (atherogenic index) usually increases in diabetic condition (the ratio was 5·3 in the DC group of rats) and this increase was countered by the experimental diet to a marked extent, namely, 1·82, which was even lower than that of the NC group (2·6). Such a decrease was seen in normal rats also as a result of SCM feeding. Significantly lower serum TAG levels ($P<0·001$) were observed (62 % lower) in the DE group as compared to those of the DC group (Table 2). An interesting observation is that as increase in HDL-cholesterol, and decreases in TAG, LDL + VLDL-cholesterol and LDL + VLDL-cholesterol:HDL-cholesterol ratio were also apparent in normal rats fed SCM.

Attenuation of urinary metabolites

Urine volumes from different groups of rats presented in Fig. 2(a) indicate that diabetic rats excreted more than sixfold higher volumes of urine compared with the non-diabetic rats throughout the experimental period. The urine volume excreted by the rats maintained on the experimental diet (DE group) decreased progressively. The urinary excretion of various metabolites such as glucose, protein, urea and creatinine was monitored at weekly intervals. Urinary excretion of glucose by diabetic rats remained high throughout the experimental period. Besides excreting a lower urine volume, the DE group of rats also showed a significant reduction in glucosuria (4·5 g/24 h at the end of 6 weeks compared with the initial 11 g/24 h) (Fig. 2(b)), and this was significantly lower than for the corresponding diabetic controls.

Table 1. Effect of dietary finger millet seed coat matter on blood urea, creatinine and protein in diabetic rats
(Mean values with their standard errors, *n* 8)

					Protein							
	Urea (mg/l)		Creatinine (mg/l)		Total (g/l)		Albumin (g/l)		Globulin (g/l)		Albumin:globulin ratio	
Rat group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NC	232[a]	23·9	8·8[a]	0·8	75[a]	3·6	38[a]	3·5	37[a]	4·2	1·00[a]	0·09
NE	217[a]	25·0	7·6[a]	0·6	73[a]	2·3	35[a]	2·0	38[a]	2·8	0·90[a]	0·05
DC	568[b]	39·0	14·0[b]	1·1	44[b]	3·2	19[b]	2·5	25[b]	3·0	0·76[b]	0·10
DE	463[c]	27·0	8·9[a]	1·2	57[c]	5·3	28[c]	3·2	29[a,b]	3·5	0·97[a]	0·11

NC, normal control diet; NE, normal experimental diet; DC, diabetic control diet; DE, diabetic experimental diet.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).

British Journal of Nutrition

Table 2. Influence of dietary finger millet seed coat matter on blood lipid profile and atherogenic index in diabetic rats (Mean values with their standard errors, *n* 8)

	Cholesterol									
	Total (mg/l)		LDL + VLDL (mg/l)		HDL (mg/l)		(LDL + VLDL): HDL		TAG (mg/l)	
Rat group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NC	522[a]	47·0	377[a]	29·2	145[a]	15·1	2·60[a]	0·20	811[a]	39·8
NE	530[a]	15·7	291[b]	23·1	239[b]	29·1	1·22[b]	0·08	502[b]	47·5
DC	958[b]	94·0	806[c]	52·1	152[a]	28·2	5·30[c]	0·34	1613[c]	145·0
DE	553[a]	51·0	357[a,b]	40·4	196[a,b]	26·3	1·82[d]	0·21	612[b]	64·0

NC, normal control diet; NE, normal-experimental diet; DC, diabetic control diet; DE, diabetic experimental diet.
[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).

Fig. 3(a) shows that the DE group of rats excreted lesser urinary protein (15 g/24 h) compared with the DC group (28 g/24 h) of rats ($P<0·001$). Likewise, Fig. 3(b) shows that the diabetic rats excreted about 15-fold higher urea (249 mg/24 h) compared with the NC group of rats (22 mg/24 h). Urinary urea decreased significantly from 230 mg/24 h (initial) to 132 mg/24 h (at the end of the experimental period) in the DE group of rats ($P<0·001$). Similarly, the DE group of rats excreted significantly lower levels of urinary creatinine (the levels decreased from the initial 27 to 18 mg/24 h at the end of the experimental period). The values were significantly lower compared with the DC group of rats ($P<0·01$) (Fig. 3(c)).

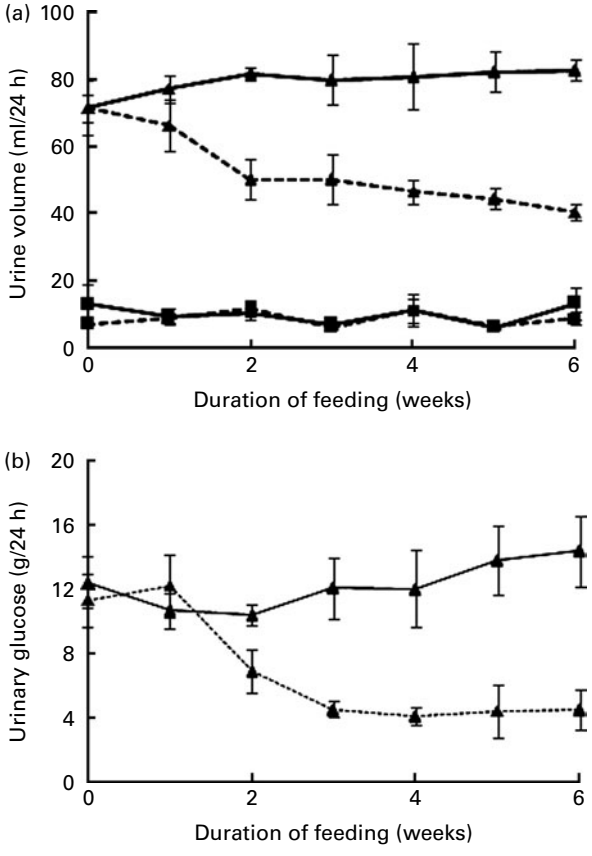

Fig. 2. Influence of dietary finger millet on (a) urine volumes and (b) glucosuria in diabetic rats. Urinary glucose was <4 mg/24 h in the normal control diet (NC, −■−) and normal experimental diet (NE, --■--) groups. DC, diabetic control diet (−▲−); DE, diabetic experimental diet (--▲--).

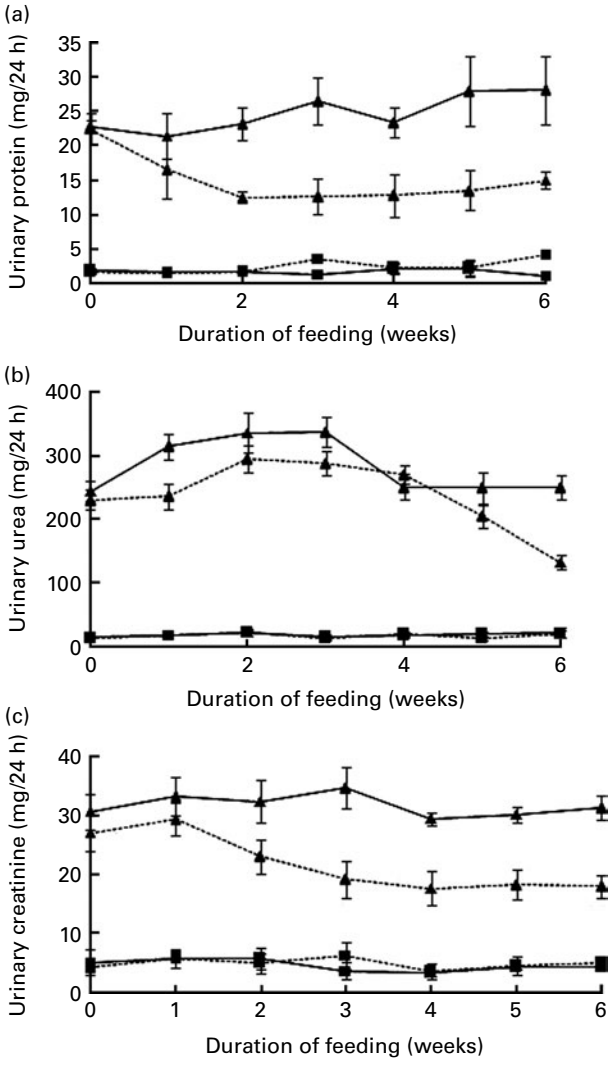

Fig. 3. Influence of dietary finger millet seed coat matter on urinary excretion of (a) protein, (b) urea and (c) creatinine in diabetic and normal rats. NC, normal control diet (−■−); NE, normal experimental diet (--■--); DC, diabetic control diet (−▲−); DE, diabetic experimental diet (--▲--).

Table 3. Influence of dietary finger millet seed coat matter on glycosylated Hb (HbA1c) levels, serum advanced glycation end product (AGE) fluorescence spectra, kidney weight and glomerular filtration rate (GFR) in diabetic and normal rats maintained on control and experimental diets

(Mean values with their standard errors, *n* 8)

	HBA1c (%)		Fluorescence intensity of AGE at 425 nm		Kidney wt (g/100 g body wt)		GFR (ml/min)	
Rat group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NC	3·25[a]	0·20	14·97[a]	0·42	0·67[a]	0·02	0·159[a]	0·05
NE	3·20[a]	0·15	13·47[a]	0·71	0·68[a]	0·04	0·141[a]	0·03
DC	6·31[b]	0·75	20·47[b]	0·86	1·59[b]	0·15	7·33[b]	0·33
DE	4·21[c]	0·66	16·46[c]	0·47	1·25[c]	0·14	2·69[c]	0·19

NC, normal control diet; NE, normal-experimental diet; DC, diabetic control diet; DE, diabetic experimental diet.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).

Lowered levels of glycosylated Hb and advanced glycation end products

The HbA1c level in diabetic control rats was 6·3 % as compared to 3·25 % in normal rats (Table 3). The HbA1c level of the DE group (4·2 %) was 33 % lower ($P<0·001$) than that of the DC group of rats. Serum AGE fluorescence spectra (Table 3) revealed that the AGE levels in the diabetic rats were higher compared with the normal rats. It was also observed that the serum AGE level of the DE group of rats was lower by 20 % compared with the level observed in the DC group of rats.

Protective effect on eye lenses

Slit lamp examination of the rat eyes was conducted to observe the cataract-associated changes in the eye lens. The incidence of mature cataract was considerably lower in the diabetic rats maintained on the experimental diet with only 10 % of the DE group of rats having developed mature cataract compared with the DC group of rats (90 % of the DC group exhibited mature cataract) at the end of the experimental period. The observations clearly revealed the presence of very mild lenticular opacity and posterior subcapsular cataract (immature cataract) in the DE group of rats in contrast to the significant lenticular opacity (mature cataract) and corneal vacuolisation observed in the DC group of rats. No lenticular opacity was observed in the non-diabetic rats (Fig. 4). These observations indicated that the SCM constituents delay cataractogenesis in diabetic animals. In order to get further insight into the mechanism of anti-cataract action of the millet SCM, activity of AR, a key enzyme involved in the aetiology of complications in diabetes, such as nephropathy-directed cataractogenesis, was determined in the eye lenses. The AR activity was lower by 25 % in the DE group as compared with that of the DC group (Fig. 5).

Amelioration of kidney pathology

Kidney weights of the diabetic rats were about 2·4-fold higher than those of the non-diabetic controls (Table 3), therefore indicating nephromegaly. The DE group of rats exhibited a 21 % lower kidney weight (1·25 g/100 g body weight) compared to that of the DC rats (1·59 g/100 g body weight). GFR of the DC group was markedly high (7·3 ml/min) compared with that of the NC group (0·2 ml/min) and among the DE and DC groups (Table 3), the GFR of the DE group (2·7 ml/min) was significantly lower ($P<0·001$). Histological examination of the sections of kidney revealed shrunken glomeruli in the DC group of rats, while glomeruli were nearly normal in the DE group (Fig. 6). Moreover, normal glomerular, tubular structures and absence of mucopolysaccharide depositions were observed in the kidney sections of the DE group of rats (Fig. 7), while mucopolysaccharide depositions and a higher degree of tubular clarifications or vacuolisation were observed in the kidney sections of the DC group.

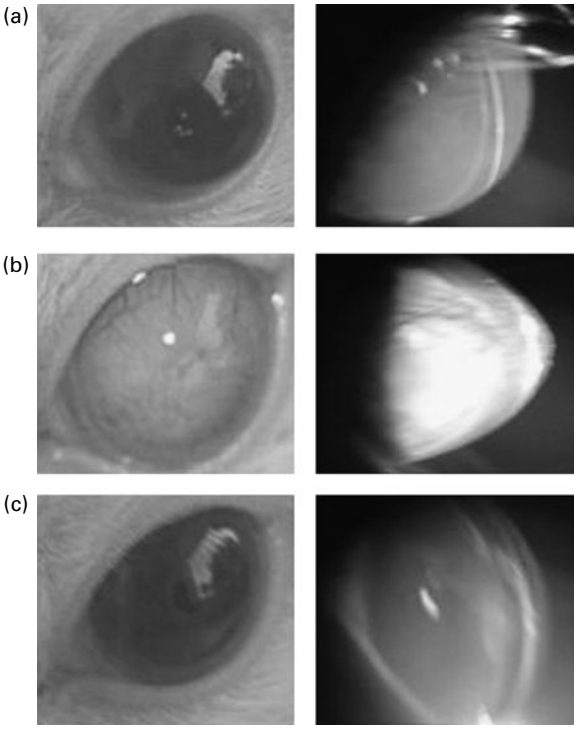

Fig. 4. Influence of dietary finger millet seed coat matter on the cataractogenesis and lenticular opacity of the eye lens in diabetic rats. (a) Normal eyes and absence of lenticular opacity in the normal control diet group, (b) corneal vascularisation, significant lenticular opacity, and mature cataract found in rats in the diabetic control diet group and (c) early cataract changes (immature posterior subcapsular cataract) found in the diabetic experimental diet group of rats.

British Journal of Nutrition

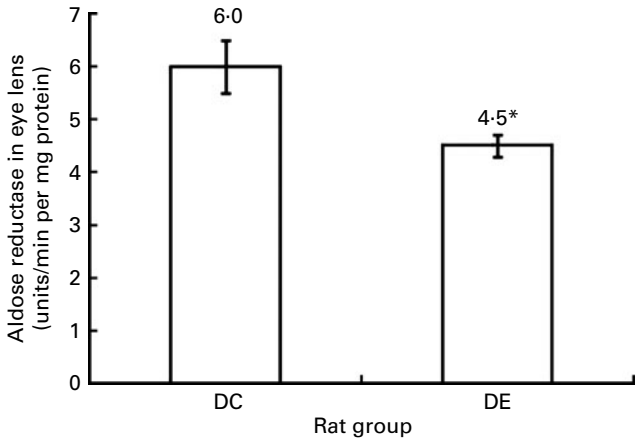

Fig. 5. Effect of dietary finger millet seed coat matter on the aldose reductase activity in the eye lens of diabetic rats. * Mean value was significantly different from that of the DC group ($P<0·05$). DC, diabetic control diet; DE, diabetic experimental diet.

Discussion

The present animal study indicated that the biochemical abnormalities associated with diabetes mellitus were significantly ameliorated by feeding a diet containing finger millet SCM. The improved metabolic status was featured by an apparent decrease in hyperglycaemia and a significant improvement in body weight. The hypoglycaemic influence of the millet SCM observed in the present study is in conformity with Hegde *et al.*[5], who observed 36 % reduction in blood glucose levels in alloxan-induced diabetic rats maintained on the millet whole meal-incorporated diet. Apart from being a rich source of dietary fibre, phytate and minerals, the millet seed coat is a reserve of many health-beneficial phenolic compounds[6,8]. It has been reported that polyphenols reduce fasting hyperglycaemia and attenuate the postprandial blood glucose response in rats[10]. *In vitro* studies with cultured cells have shown that polyphenols such as caffeic acid, epigallocatechin-3-gallate and *iso*-ferulic acid increase glucose uptake by peripheral tissues[10]. Hence, the observed health benefits in the DE group may possibly be attributed to the synergistic effect of these phenolic compounds present in the millet SCM. Lower urinary glucose excretion observed in the DE group is consistent with the blood glucose-lowering effect of the millet SCM.

Phytate is known to have amylase-inhibitory properties[25] and a regulatory role in insulin secretion from pancreatic β-cells. Earlier reports from our laboratory have shown that millet seed coat phenolics are non-competitive inhibitors of intestinal α-glucosidase and pancreatic amylase[7]. As these inhibitors are proven modulators of postprandial glycaemia, they play a significant role in the management of diabetic complications. Phenolic compounds are also known to enhance insulin activity[26], regulate intestinal GLUT[27], increase muscle glucose uptake and reduce hepatic gluconeogenesis[28]. Hence, the phytate of the SCM may have complemented the positive role of polyphenols towards regulation of postprandial glycaemia and ameliorating complications associated with diabetes via impeding glucose absorption in the small intestine.

Insoluble fibre has been reported to be effective in the glycaemic control in dogs with insulin-dependent diabetes mellitus[29]. Insoluble fibre of pre-germinated brown rice has been reported to lower both postprandial blood glucose and insulinaemic responses in normal Wistar rats[30]. Fibre concentrates of rice bran were effective in lowering serum glucose and cholesterol in both type 1 and type 2 diabetics[31]. Dietary fibre present in the millet seed coat is mostly insoluble in nature (about 92 % insoluble and 8 % soluble), and hence the anti-hyperglycaemic action of the SCM in the present study could also have been contributed by dietary fibre present in the millet SCM.

Hypercholesterolaemia and hypertriacylglycerolaemia are the usual complications in diabetes mellitus[32]. Atherosclerosis is a serious consequence of long-term diabetes and is the major cause of death in diabetics. In the present study, the diabetic rats exhibited significant elevations of serum cholesterol, TAG and atherogenicity index. These lipid and lipoprotein

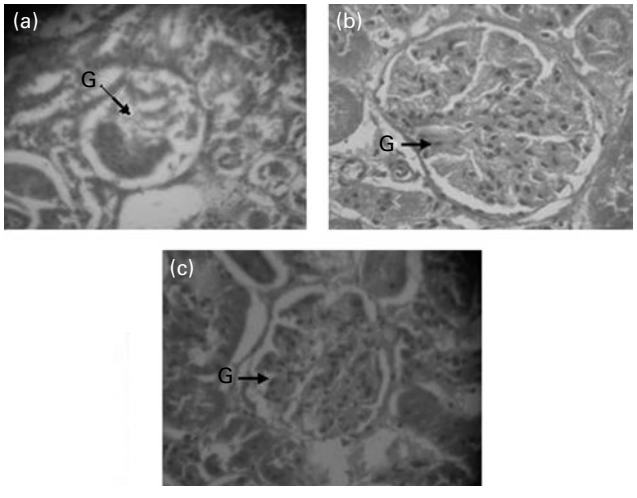

Fig. 6. Effect of finger millet seed coat matter feeding on kidney glomerular structures in diabetic rats. (a) Normal glomerulus (G) in the normal control diet group; (b) shrunken glomerulus in the diabetic control diet group and (c) nearly normal glomerulus in the diabetic experimental diet group.

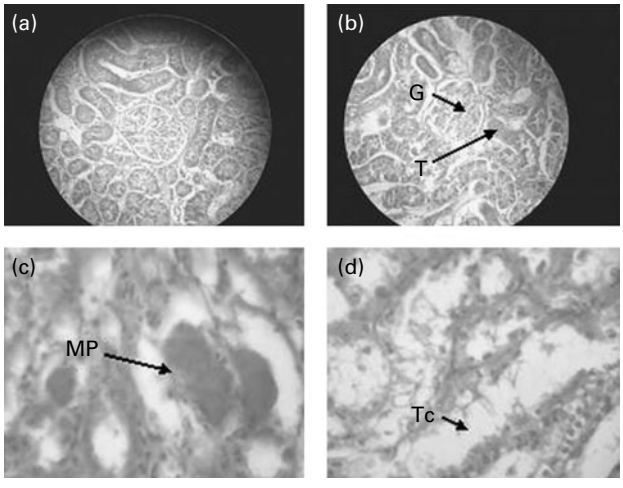

Fig. 7. Effect of dietary finger millet seed coat matter on kidney histology in diabetic rats. (a) Mucopolysaccharide depositions in the diabetic control diet (DC) group; (b) vacuolisation in kidney tubules (T) in the DC group; (c) normal glomerulus and tubules in the normal control diet group and (d) kidney section showing nearly normal glomerulus, proximal tubules without mucopolysaccharide depositions. G, glomerulus; MP, mucopolysaccharide deposition; Tc, tubular clarifications.

abnormalities developed in the diabetic condition were significantly countered by dietary SCM. Cholesterol-lowering effects of finger millet whole meal in normal rats[33] and of finger millet as well as kodo millet in alloxan-induced diabetic rats[5] have been reported. The lipid-lowering effect of the millet SCM may be attributed to its dietary fibre and polyphenol constituents. Reduction in blood glucose in diabetic rats fed with a 10 % wheat bran-containing diet has been reported[34]. Several reports on the hypoglycaemic and hypocholesterolaemic properties of the polyphenols isolated from cereals are available[35]. Hence, the lower lipid levels and atherogenic index observed in the DE group of rats may be partly attributed to the anthocyanin contents of the millet SCM.

Diabetic rats maintained on the millet SCM diet exhibited higher levels of serum albumin and decreased serum urea levels which were accompanied by decreased excretion of protein and urea compared with the DC group of rats. This indicates a lesser degree of protein catabolism in the DE group of rats. Serum creatinine is widely interpreted as a measure of the GFR and used as an index of renal function in clinical practice. Lower levels of blood and urinary creatinine and attenuated nephromegaly in the DE group of rats are suggestive of improved renal function in this group of rats, which was also complemented by the histopathological picture of the kidneys in experimental rats.

Chronic hyperglycaemia plays an important role in the pathogenesis of vascular complications leading to atherosclerosis, a variety of neuropathies, cataract and the end-stage renal failure[36]. Diabetic cataract is characterised by opacification of the lens, eventual loss of vision that occurs at a much earlier age than senile cataract. Three possible mechanisms involved in cataract formation as a result of hyperglycaemia are the polyol pathway, oxidation and non-enzymic glycation[37]. In the polyol pathway, glucose is converted into sorbitol by AR and, subsequently, sorbitol is oxidised to fructose by sorbitol dehydrogenase. In diabetes mellitus, the increased glucose level results in sorbitol being produced at a faster rate than its oxidation to fructose, and as a result, sorbitol accumulates in blood vessels, nerves, retina and kidney. Accumulated sorbitol can produce a hyperosmotic effect, leading to membrane permeability changes and the onset of cellular pathology[38]. Glycation of lens protein crystallin may cause conformational changes resulting in exposure of thiol groups to oxidation and cross-link formation[39]. The lens crystallins readily accumulate as AGE, which in turn cause aggregation of the lens crystallins producing the high-molecular-weight material responsible for opacification[40]. The millet SCM, being a rich source of phenolic compounds with antioxidant properties, may delay cataractogenesis in the diabetic rats. The AR-inhibitory property of the millet seed coat polyphenols *in vitro* has been previously reported[41]. Thus, the enzyme-inhibitory property of the millet seed coat polyphenols may also contribute to the anti-cataractogenic property.

Lowered levels of AGE, HbA1c, AR activity and lesser lenticular opacity as well as the absence of mature cataract in the DE group of rats indicate that the millet SCM prevents protein glycation and delays cataractogenesis in diabetic rats. Reduced rat tail tendon collagen glycation has been reported in diabetic rats maintained on a millet diet[5]. Hence, the anti-cataractogenic properties of the millet SCM may partly be attributed to the enzyme-inhibitory as well as to the anti-protein glycation properties of the millet seed coat phenolic compounds. Protein glycation and AGE formation are closely associated with artherosclerosis. Glycation and AGE formation in diabetes are also accompanied by increased oxidation of LDL, resulting in an increase in the atherogenic oxidised LDL[42]. Hence, the lower atherogenic index observed in the diabetic rats maintained on the SCM diet may also be partly attributed to the anti-glycation properties of millet seed coat phenolics.

Diabetic nephropathy is characterised by the thickening of the basement membrane, expansion of the mesangium, reduced filtration, albuminuria and ultimately renal failure. AGE have been detected in renal tissues in amounts that correlate with the severity of diabetic nephropathy[43]. Accumulation of AGE on collagen in the basement membrane together with their ability to trap plasma proteins may also contribute towards thickening of the basement membrane as well as altered filtration and ultimately loss of glomerular function[44]. Normal glomeruli and absence of mucopolysaccharide depositions in the kidney tissues of the DE group of rats may possibly be due to the improved glycaemic status and lowered hyperglycaemia-induced protein glycation.

Conclusions

The present animal study revealed that finger millet SCM caused significant hypoglycaemic, hypocholesterolaemic and nephroprotective properties, and delayed cataractogenesis in streptozotocin-induced diabetic rats. Hence, the millet SCM matter can be utilised as a functional ingredient for the development of functional foods for diabetics to derive beneficial effects in the regulation of glucose homeostasis and prevention of dyslipidaemia, thus helping manage diabetes and its complications. The health-beneficial properties of the millet SCM can be attributed to its dietary fibre, phytate and phenolic constituents.

Acknowledgements

The first author (S. S.) is thankful to the Council of Scientific and Industrial Research, New Delhi, for the award of senior research fellowship by India. The financial support from All India Co-ordinated Small Millets Improvement Project of Indian Council of Agricultural Research, New Delhi, is gratefully acknowledged. The services of Dr Surendhra (Head, Ophthalmology Department, K.R Hospital, Mysore) in slit lamp examination of rat eyes are gratefully acknowledged. There are no conflicts of interest whatsoever among the authors. S. S. and M. R. H. were responsible for the bench work. K. P. supervised the whole animal experiment and analytical work. K. S. handled planning of the animal experiment, data interpretation and writing of the manuscript. N. G. M. is the project leader, who was responsible for the planning of this research activity.

References

1. Wild S, Roglic G, Green A, *et al.* (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* **27**, 1047–1053.

2. Srinivasan K (2008) Fenugreek and traditional antidiabetic herbs of Indian origin. In *Nutraceuticals, Glycemic Health and Type 2 Diabetes*, pp. 311–378 [V Pasupuleti and J Anderson, editors]. Ames, IA: Blackwell Publishing Co.
3. Jensen MK, koh-Banerjee P, Franz M, *et al.* (2006) Whole grains, bran, and germ in relation to homocysteine and markers of glycemic control, lipids and inflammation. *Am J Clin Nutr* **83**, 275–283.
4. Liu S, Stampfer MJ, Hu FB, *et al.* (1999) Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* **70**, 412–419.
5. Hegde PS, Rajasekaran NS & Chandra TS (2005) Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced diabetic rats. *Nutr Res* **25**, 1109–1120.
6. Chethan S & Malleshi NG (2007) Finger millet polyphenols: optimization of extraction and the effect of pH on their stability. *Food Chem* **105**, 862–870.
7. Shobana S, Sreerama YN & Malleshi NG (2009) Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of α-glucosidase and α-amylase. *Food Chem* **115**, 1268–1273.
8. Shobana S, Meera MS & Malleshi NG (2006) Major nutrients and phytochemical contents of the finger millet fractions, Sovenier, pp. 26. Hyderabad: Indian Convention of Food Scientists and Technologists, AFST(I).
9. Thompson LU, Button CL & Jenkins DJA (1987) Phytic acid and calcium effect *in vitro* rate of navy bean starch digestion and blood glucose response in humans. *Am J Clin Nutr* **46**, 467–473.
10. Scalbert A, Manach C, Morand C, *et al.* (2005) Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* **45**, 287–306.
11. AACC (2000) *Moisture (Method 44-19), Protein (Method 46-12), Fat (Method 30-25), Ash (Method 8-01)*. St Paul, MN: American Association of Cereal Chemists.
12. Asp NG, Johansson CG, Hallmer H, *et al.* (1983) Rapid enzymatic assay of insoluble and soluble dietary fibre. *J Agric Food Chem* **31**, 476–482.
13. Singleton VL, Orthofer R & Lamuela-Raventos RM (1995) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. *Methods Enzymol* **299**, 152–171.
14. Huggett ASG & Nixon DA (1957) Use of glucose oxidase, peroxidase and *o*-dianisidine in the determination of blood and urinary glucose. *Lancet* **273**, 368–370.
15. Wybenga DR, Di-Giorgio J & Pileggi VJ (1971) Manual and automated methods for urea nitrogen measurement in whole serum. *Clin Chem* **17**, 891–895.
16. Oser BL (1965) *Hawk's Physiological Chemistry*, 14th ed., pp. 1040. New York: Tata McGraw Hill, Inc.
17. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.
18. Cooper GR (1972) *Standard Methods of Clinical Chemistry*, vol. 7, pp. 175. New York: Academic press.
19. Searcy RL & Bergquist LM (1960) A new color reaction for quantitation of serum cholesterol. *Clin Chim Acta* **5**, 192–197.
20. Warnick GR & Albers JJ (1978) A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high-density lipoprotein-cholesterol. *J Lipid Res* **19**, 65–76.
21. Fletcher MJ (1968) A colorimetric method for estimating serum triglycerides. *Clin Chim Acta* **22**, 393–397.
22. Kim HY & Oh JH (1999) Screening of Korean forest plants for rat lens aldose reductase inhibition. *Biosci Biotech Biochem* **63**, 184–188.
23. Monnier VM & Cerami A (1981) Nonenzymatic browning *in vivo*: possible process for aging of long-lived proteins. *Science* **211**, 491–493.
24. Muruganandan S, Srinivasan K, Gupta S, *et al.* (2005) Effect of mangiferin on hyper glycemia and atherogenicity in streptozotocin diabetic rats. *J Ethnopharmacol* **97**, 497–501.
25. Knuckles BE & Betschart AA (1987) Effect of phytate and other myo-inositol phosphate esters on α-amylase digestion of starch. *J Food Sci* **52**, 719–721.
26. Anderson RA & Polansky MM (2002) Tea enhances insulin activity. *J Agric Food Chem* **50**, 7182–7186.
27. Shimizu M, Kobayashi Y, Suzuki M, *et al.* (2000) Regulation of intestinal glucose transport by tea catechins. *Biofactors* **13**, 61–65.
28. Liu IM, Hsu FL, Chen CF, *et al.* (2000) Antihyperglycemic action of *iso*-ferulic in streptozotocin induced diabetic rats. *Br J Pharmacol* **129**, 631–636.
29. Kimmel SE, Michel KE, Hess RS, *et al.* (2000) Effects of insoluble and soluble dietary fibre on glycemic control in dogs with naturally occurring insulin-dependent diabetes mellitus. *J Am Vet Assoc* **216**, 1076–1081.
30. Seki T, Nagase R, Torimitsu M, *et al.* (2005) Insoluble fibre is a major constituent responsible for lowering the postprandial blood glucose concentration in the pre-germinated brown rice. *Biol Pharmaceut Bull* **28**, 1539–1541.
31. Qureshi AA, Sami SA & Khan FA (2002) Effects of stabilized bran, its soluble and fiber fractions on blood glucose levels and serum lipid parameters in humans with diabetes mellitus types I and II. *J Nutr Biochem* **13**, 175–187.
32. Howard BV & Howard WJ (2005) Pathophysiology and treatment of lipid disorders in diabetes. In *Joslin's Diabetes Mellitus*, 14th ed., pp. 563–584 [R Khan, GC Weir, GL King, AM Jacobson, AC Moses and RJ Smith, editors]. New York: Lippincott Williams and Wilkins.
33. Pore MS & Magar NG (1976) Effect of ragi feeding on serum cholesterol level. *Indian J Med Res* **64**, 909–914.
34. Nandini CD, Sambaiah K & Salimath PV (2000) Effect of dietary fiber on intestinal and renal dissacharidases in diabetic rats. *Nutr Res* **20**, 1301–1307.
35. Tsuda T, Horio F, Uchida K, *et al.* (2003) Dietary cyanidin 3-*o*-β-D-glucoside rich purple corn colour prevents obesity and ameliorates hyperglycemia in mice. *J Nutr* **133**, 2125–2130.
36. Yamagishi S & Imaizumi T (2005) Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. *Curr Pharm Des* **11**, 2279–2299.
37. Spector A (1995) Oxidative stress induced cataract: mechanism of action. *FASEB J* **9**, 1173–1182.
38. De la Fuente JA & Manzanaro S (2003) Aldose reductase inhibitors from natural sources. *Nat Prod Rep* **20**, 243–251.
39. Ansari NH, Awasthi YC & Srivastava SK (1980) Role of glycosylation in protein disulphide formation and cataractogenesis. *Exp Eye Res* **31**, 9–19.
40. Perry RE, Swamy MS & Abraham EC (1987) Progressive changes in lens crystallin glycation and high molecular weight aggregate formation leading to cataract development in streptozotocin-diabetic rats. *Exp Eye Res* **44**, 269–282.
41. Chethan S, Dharmesh MS & Malleshi NG (2008) Inhibition of aldose reductase from cataracted eye lenses by finger millet (*Eleusine coracana*) polyphenols. *Bioorg Med Chem* **16**, 10085–10090.
42. Ahmed N (2005) Advanced glycation end products role in pathology of diabetic complications. *Diabetes Res Clin Pract* **67**, 3–21.
43. Sugiyama S, Miyata T, Horie K, *et al.* (1996) Advanced glycation end-products in diabetic nephropathy. *Nephrol Dial Transplant* **14**, Suppl. 3, 1–9.
44. Monnier VM, Sell DR, Nagaraj RH, *et al.* (1992) Maillard reaction-mediated molecular damage to extracellular matrix and other tissue proteins in diabetes, aging and uremia. *Diabetes* **41**, Suppl. 2, 36–41.

British Journal of Nutrition