ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF *ELEUCINE CORACANA* (L.) GEARTN. (FINGER MILLET) SEED COAT MATTER IN STREPTOZOTOCIN INDUCED DIABETIC RATS

1 Okoyomoh K., 2 Okere O.S., 3 Olowoniyi O.D. and 2* Adejo G.O.

1 Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria.
2 Department of Biochemistry, Faculty of Science and Technology, Bingham University, Nasarawa State, Nigeria.
3 Department of Science Laboratory Technology, School of Applied Science, Nasarawa Federal Polytechnic, Nasarawa State, Nigeria.

Accepted November 28, 2013

Herbal drugs are traditionally used in various parts of the world to cure different diseases. The present study evaluates the antioxidant and antidiabetic properties of seed coat matter (SCM) of grains of black finger millet (*Eleusine coracana* (L.) Geartn.). 20% and 40% in streptozotocin (STZ) induced diabetic rats. *Eleusine coracana* exhibited significant antidiabetic activity resulting to a 45% reduction in the diabetic experimental group treated with 40%SCM. Feeding the experimental rats with various concentration of the SCM exhibited significant protective effect by lowering the serum levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Catalase (CAT) and superoxide dismutase (SOD) activities were increased while the concentration of thiobarbituric acid reactive substances (TBARS) was significantly lowered. Histo-pathological observations also revealed that SCM of *Eleusine coracana* offered protection to the animals against pancreatic, kidney and liver STZ induced damages. The result indicates that the various concentrations of *Eleusine coracana* grains possess antioxidant and antidiabetic potentials in STZ induced diabetic rats.

Key words: Finger millet, Hyperglycaemia, Seed coat matter, Streptozotocin.

Abbreviations: AGE, advanced glycation end product; DC, diabetic control; DE, diabetic experimental; NC, normal control; SCM, seed coat matter; STD DRG, standard drug.

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that presently affects 200 million people of the world’s population (Wais et al., 2012). Current figures indicate that people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030, if no urgent action is taken (IDF, 2011). International diabetic federation also estimates that as many as 183 million people are unaware that they have diabetes (IDF, 2011). While global incidence of diabetes is increasing in an exponential manner, it has been shown that the consumption of foodstuffs containing complex carbohydrates with high level of dietary fibre and health benefiting phytochemicals like polyphenols...
and phytates (Bouchenak and Lamri-Senhadji, 2013); Pulse Canada, 2013), could improve the condition (Shobana and Malleshi, 2007). Also, the consumption of whole grains has been associated with lower risk of diabetes (Anderson, 2004) and cardiovascular diseases (CVD) (Liu et al., 1999). Whole grain cereals form the most important sources of dietary fibre, minerals and phytochemicals with antioxidant activity (Anderson et al., 2000). 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 (Aparicio-Fernandez et al., 2005; Reynoso-Camacho et al., 2006).

*E. coracana* also known as ‘African finger millet' belongs to the family Poaceae (Gramineae) A. L. Jussieu, is an annual plant widely grown as a cereal in the arid areas of Africa and Asia. The millet seed coat reserves several phenolic compounds like phenolics, flavonoids, polymeric tannins and anthocyanins, some of which are effective inhibitors of pancreatic amylase and intestinal α-glucosidase (Chethan and Malleshi, 2007). It is also a rich source of phytates and minerals (Shobana et al., 2006). Traditionally, finger millet food preparations are known for their higher sustaining power, lower glycaemic response and higher satiety scores compared with other cereal foods which are usually recommended for diabetic patients. Dietary polyphenols and phytates are known for their ability to reduce carbohydrate digestibility and thereby regulate postprandial glycaemic response (Thompson et al., 1987). Moreover, polyphenols are known to inhibit glucose absorption and prevent advanced glycation end product (AGE) formation (Scalbert et al., 2005). 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.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

All assays kits were from Randox laboratories Ltd. Ardmore, Co. Antrim UK. Streptozotocin (STZ) was purchased from sigma chemical (St Louis U.S.A). All other chemicals and solvents used were of analytical grade.

**Identification and preparation of finger millet seed coat matter (SCM)**

Seeds of Black Finger Millet (*E. coracana*) were collected from Institute of Agricultural Research (IAR), Faculty of Agriculture, ABU Zaria, Nigeria. The seeds were identified at the Herbarium unit, Department of Biological sciences ABU, Zaria, with voucher #: 2843. SCM was prepared from finger millet according to the protocol described previously by (Chethan and Malleshi, 2007). SCM and basal diet (Vital Feed Nigeria Ltd) were combined in the ratios of 1:5 and 2:5 and thoroughly mixed.

**Animal Collection**

Male Wistar Strain albino rats weighing between 150-250g obtained from the animal house of the Department of pharmaceutical science, ABU Zaria were used. The rats were kept and maintained in well ventilated cages under ambient environmental conditions. They were maintained on grower’s mash (Vital feeds Nigeria Ltd) and provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for two weeks before the experiment.

**Induction of Diabetes**

Diabetes mellitus was induced by single injection (*i.p.*) of 55mg/kg of Streptozotocin, dissolved in 0.1M fresh cold citrate buffer (pH 4.5) into 12h fasted rats (Burcelin et al., 1995). After 3 days of STZ post-injection, the blood sugar levels were determined with a glucometer (Acc-cheek Advantage Roche diagnostics GmbH, Germany). Rats with fasting blood glucose levels >126mg/dl (11.1mmol/L) were considered diabetic hence, selected for experimentation.

**Animal grouping and treatment**

A division of 5 groups made of 5 rats each were
used in this study. Group 1: Diabetic rats without treatment (Diabetic control, DC). Group 2: Diabetic rats treated daily with Metformin (2.5mg/kg body weight) daily (DE+STD DRG) Group 3: Diabetic rats fed with 20% finger millet SCM (DE+20%). Group 4: Diabetic rats fed with 40% finger millet SCM (DE+40%). Group 5: Non-diabetic and untreated (Normal control, NC).

Biochemical analysis

Rats were fed with SCM-incorporated diet daily for six (6) weeks. Bodyweights were recorded weekly using standard analytical weighing balance. Fasting blood glucose level was determined using the method described by Clark et al. (1962). Rats were fasted overnight, anesthetized with chloroform and sacrificed 24h after the last treatment. Serum was harvested from the collected blood and used to assay for AST, ALT, ALP, total protein, urea and creatinine. Serum was also used for the estimation of CAT and SOD activities, and TBARS concentration. Histo-pathological examination was done using the method described by Bancroft and Gamble (2008).

Statistical analysis

Results were presented as mean ± SEM, ANOVA was carried out using the SPSS program (version 16 SPSS Inc, Chicago, USA) and the Duncan multiple range test was used to test the differences between groups. P value was set at 0.05 (Duncan et al., 1955).

RESULTS

Body weight changes

Table 1 showed that the relative body weight in NC group was significantly (P<0.05) higher than the diabetic control group. A significant weight loss was observed in the DC rats group (P<0.05). However, similar to rats group treated with standard drug-Metformin, diabetic rats maintained on experimental diet (20% and 40% SCM) showed significant improvement in body weight (in a dose-dependent manner though), throughout the experimental duration as compared to the DC group.

Blood glucose levels changes

Figure 1 showed significant (P<0.05) reduction in the blood glucose levels in the experimental groups (DE+40% or 20% SCM) when compared to the DC group. But the DE+40% SCM group showed higher ameliorative effect than DE+20% SCM group. Also, it was observed that while the mean value of the initial and final fasting blood glucose concentration of the DC group of rats did not change appreciably throughout the experiment, there was significant (P<0.05) reduction in the group treated with standard drug (Metformin).

Attenuation of liver enzymes activities

There was significantly higher (p<0.05) serum AST activity in the DC group than the experimental
Table 2. ALT, AST and ALP activities in rats fed with various concentration of finger millet SCM.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST(U/I)</th>
<th>ALT(U/I)</th>
<th>ALP(U/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>26.64±1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.67±1.86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>84.33±4.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE+STD DRUG</td>
<td>22.00±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.67±0.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.00±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE+20% SCM</td>
<td>22.00±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.00±1.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.67±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE+40% SCM</td>
<td>21.33±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.33±1.45&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>63.33±6.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NC</td>
<td>20.66±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.81±1.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>57.67±4.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n=5). No significant difference between values with the same superscript down the column (p<0.05).

Table 3. Creatinine and urea concentrations in rats fed with various concentration of finger millet SCM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (nMol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>2.60±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.40±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE+STD DRUG</td>
<td>1.23±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE+20% SCM</td>
<td>1.20±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.63±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DE+40% SCM</td>
<td>1.47±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.50±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NC</td>
<td>0.55±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.83±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n=5). No significant difference between values with the same superscript down the column (p<0.05).

Groups (DE+20% and 40% SCM) (Table 2). Group fed with 40% SCM presented no significant (p<0.05) difference compared with 20% SCM group or the group treated with 2.5mg/kg of Metformin. The DC group was significantly higher (p<0.05) in AST activity than all other groups. Also, the ALT and ALP activities among the various groups presented similar observations as found in AST activity (Table 2).

**Amelioration of kidney pathology**

Serum creatinine concentration (Table 3) in DC group showed significantly higher (P<0.05) values compared to the NC group. There was no significant difference among the experimental groups (DE+20% or 40% SCM) and the group treated with standard drug (2.5mg/kg of Metformin). However, groups treated with SCM or standard drug presented significantly higher (P<0.05) creatinine concentrations than the NC group. Urea concentration among the various groups also presented similar observations as found in creatinine concentration (Table 3).

**Improved anti-oxidant enzyme activity**

TBARS concentration was significantly higher (p<0.05) in the DC group than the NC, experimental (DE+20% and 40% SCM) and DE+STD DRUG groups (Table 4). Apart from the DC group, there was no significant difference among all the other groups. On the contrary, SOD and CAT values showed significantly lower (p<0.05) activities in the DC group than the NC, experimental (DE+20% and 40% SCM) and DE+STD DRUG groups (Table 4). Also, apart from the DC group, there was no significant difference among all the other groups.
Table 4. SOD, TBARS and CAT activity in rats fed with various concentration of finger millet SCM.

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS (nmol/mg protein)</th>
<th>SOD (U/ml)</th>
<th>CAT (Umol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>2.46±0.22(^a)</td>
<td>0.90±0.20(^b)</td>
<td>32.00±0.58(^a)</td>
</tr>
<tr>
<td>DE+STD DRUG</td>
<td>1.63±0.15(^a)</td>
<td>2.00±0.06(^c)</td>
<td>49.33±2.40(^c)</td>
</tr>
<tr>
<td>DE + 20% SCM</td>
<td>1.66±0.12(^a)</td>
<td>1.76±0.09(^c)</td>
<td>41.33±2.60(^a)</td>
</tr>
<tr>
<td>DE +40% SCM</td>
<td>1.80±0.26(^a)</td>
<td>1.60±0.10(^c)</td>
<td>45.66±2.73(^c)</td>
</tr>
<tr>
<td>NC</td>
<td>1.63±0.08(^a)</td>
<td>1.60±0.20(^c)</td>
<td>41.00±1.15(^a)</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (n=5). No significant difference between values with the same superscript down the column(p<0.05).

Figure 1. Effect of dietary finger millet SCM on blood glucose levels of STZ induced diabetic rats.

Histopathological study of the pancreas, kidney and liver

In the histo-pathological studies of the pancreatic tissues of the rats, Plates 2 and 3 (Figure 2) showed slight regeneration of islet cells as compared to plate 4 (Figure 2) which is the DC that showed necrosis of islet cells. Among the group treated diabetic groups, the DE+STD DRG group Plate 5 (Figure 2) showed the most regeneration of islet cells. Histopathological studies of kidney tissues of rats showed intense glomerular necrosis in the DC group as shown in plate 4 (Figure 3) as compared to the NC in Plate 1 (Figure 2) where there was no necrosis. In the experimental groups (DE+20% and 40%) SCM, the former showed more glomerular necrosis as seen in Plates 2 and 3 (Figure 3). Slight tubular necrosis seen in the DE+40% SCM group was comparable to the DE+STD DRG group as seen in Plates 3 and 5 (Figure 3) slides, respectively. The liver tissues histopathological studies showed that DC group presented hepatic necrosis, contrary to the NC where normal hepatic cells were observed, in plates 4 and 1 (Figure 4), respectively. But while the DE+STD DRG group showed hepatic necrosis similar to the DC group, the experimental groups (DE+20% and 40%) SCM showed only slight hepatic necrosis as observed in Plates 5, 2 and 3 (Figure 4), respectively.

DISCUSSION

The animal study indicated that the biochemical abnormalities associated with diabetes mellitus were significantly improved by feeding a diet containing finger millet SCM. The improved metabolic status was featured by a decrease in hyperglycaemia and...
a significant improvement in body weight. The hypoglycaemic influence of the finger millet SCM observed in the present study is in conformity with Hegde et al. (2005), who observed 36% and Shobana et al., (2010) who observed 39% reduction in blood glucose levels in alloxan and streptozotocin-induced diabetic rats respectively, maintained on the millet whole meal-incorporated diet. Apart from being a rich source of dietary fibre, phytates and minerals, the millet seed coat is a reserve of many health-beneficial phenolic compounds (Shobana et al., 2006; Chethan and Malleshi, 2007). It has been reported that polyphenols reduce fasting hyperglycaemia and attenuate the postprandial blood glucose response in rats (Scalbert et al., 2005). In vitro studies with cultured cells have shown that polyphenols such as caffeic acid, epigallocatechin-3-gallate and isoferulic acid increase glucose uptake by peripheral tissues (Scalbert et al., 2005). 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.

Urea and creatinine lowering observed in the DE groups was consistent with the blood glucose-lowering effect of the finger millet SCM. Phytate is known to have amylase-inhibitory properties (Knuckles and Betschart, 1987) and a regulatory role in insulin secretion from pancreatic β-cells. Earlier reports have shown that finger millet phenolics are non-competitive inhibitors of intestinal α-glucosidase and pancreatic amylase (Shobana et al., 2009). 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 (Anderson and Polansky, 2002), regulate intestinal Glucose transporter (GLUT) (Shimizu et al., 2000), increase muscle glucose uptake and reduce hepatic gluconeogenesis (Liu et al., 2000). 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. There was significant reduction in liver enzyme activity; this could be attributed to the polyphenolic content of the seed coat matter. The reduction in urea and creatinine level when treated with various concentration of the SCM was in consistent with Shobana et al., (2010) and Hedge et al., (2005). There was relatively high activity of
Figure 3. Photomicrographs of sections of rats kidney (H & E stain x250), showing 1 = NC, 2 & 3 = DE group treated with 20% and 40% finger millet SCM respectively, 4 = DC, and 5 = DE group treated with a standard drug (metformin).

Figure 4. Photomicrographs of sections of rats liver (H & E stain x250), showing 1 = NC, 2 & 3 = DE group treated with 20% and 40% finger millet SCM respectively, 4 = DC and 5 = DE group treated with a standard drug (metformin).
endogenous enzymes, viz: SOD and CAT, and lower TBARS concentration by the SCM compared to the DC groups. These have presented a generally better protection against STZ induced diabetic rats. Chandrasekara and Shahidi (2010) showed that the potential of finger millets as natural sources of antioxidants could be due to varietal differences that exist in the contents of phenolics as well as antioxidant capacities. Chethan et al., (2007) observed the antioxidant activity of white finger millet in reducing lipid peroxidation. The experimental groups fed with different concentrations of the SCM were able to effectively promote regeneration after the necrotic damages done by the STZ to the β-cells when compared to the DC group. Also, the SCM effectively protected the liver tissues from necrotic damages due to STZ administration, showing some hepato-protective abilities as presented by the slides. Kidney tissue slides also showed nephro-protective abilities of finger millet SCM.

CONCLUSION

The present animal study showed that the biochemical abnormalities associated with diabetes mellitus were significantly ameliorated by feeding a diet containing finger millet SCM. These findings go further to show that finger millet SCM possesses significant antioxidant, anti-diabetic, anti-hyperglycemic and nephro-protective properties and thus, may be useful in the management of diabetic complications.

REFERENCE


