Effect of Pearl Millet on Glycaemic Control and Lipid Profile in Streptozocin Induced Diabetic Wistar Rat Model

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Authors’ contributions

This work was carried out in collaboration among all authors. Author KAOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RIA and HSH managed the analyses of the study. Authors AOA and EIM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was designed to investigate the effect of Pearl millet on glycaemic control and lipid profile in streptozocin diabetic rat model.

Methodology: Forty healthy mature male rats were used in this study. The rats divided into 4 groups, ten rats in each and group (A) and (B) normal control rats while group (C) and (D) considered as diabetic rats. Diabetes induced by intraperitoneal injection of 40 mg/kg streptozocin and confirmed by high blood glucose level which considered day 0. The experiment 1, included two groups (A and C), equal rats and the parameters investigated were measured in days 0, 14 and 28. The experiment 2 included two groups (B and D) were received 20% pearl millet and the blood samples were measured in days 0, 14 and 28.
1. INTRODUCTION

Diabetes is a common disease in all populations enjoying an affluent lifestyle. It is characterized by chronic hyperglycemia resulting either from insulin deficiency producing type one diabetes (insulin dependent DM) or insulin resistance producing type two diabetes (non insulin dependent DM) leading to an alteration in carbohydrate, lipid and protein metabolism. Diabetes may be primary or secondary; it's irreversible and carries long term complications [1]. According to WHO 2015 published figure 9% of the world population aged 18 and above has diabetes and an estimated 1.5 million deaths per year are attributed to diabetes directly [2].

There are many recommendations of nutrition for diabetics [3] suggest dietary regimen of plant based on food with lower meat, sweet, high fibers and refined grains as a Mediterranean diet. Which characterized by a high intake of vegetables, fruits, nuts, cereals and low intake of saturated fatty acids and amodater intake of fish. Some dietary proteins such as soy and cod proteins have been found to have a favorable effect on glycaemic response and serum adiponectin level [4,5]. Low fat diet, vegans and cereal diet were found to be effective in controlling hyperglycemia in diabetics [6].

Wistar rats are an outbreed albino rats, this breed was developed in Wistar institutes in 1906 for use in biochemical and medical researches and the typical life span is 2.5 to 3.5 years. Animal models are used to learn more about the disease, diagnosis and treatment. They are easy to handle, low cost and high rate of reproduction [7].

Pearl millet contains high iron and zinc contents which may help to increase haemoglobin level [8]. However the non-nutrient contents such as phytate and tannate and polyphenols may decrease the bioavailability of iron [9]. Pearl millet has high fiber content and helpful in dealing with the problem of constipation [8]. Pearl millet also, has a high content of antioxidants namely the phenolic compounds may have anti-cancer property [10]. Concerning allergy pearl millet is gluten-free grain and only grain that returns its alkaline property after cooking which is ideal for people with a wheat allergy. Pearl millet is also preventing the formation of excess acidity so it is recommended for curing peptic ulcers. On other hand it contains a high amount of magnesium which reduces the severity of respiratory problems such as asthma, also it has a role in bone growth and repairs due to its high phosphorous content.

Lipids play a crucial role in the development of insulin resistance and type 2 DM. adipose tissues now are well established as an endocrine organ because it releases many biologically active substances known as adipocytokines, such as adiponectin, TNF, PAI1, IL6 and resist in response to specific extracellular stimuli and to variations in metabolic conditions [11,12]. It is well documented that, adiponectin is an insulin sensitizing hormone and plasma adiponectin levels are highly associated with insulin sensitivity in type 2 DM. Plasma adiponectin levels decrease with the development of obesity in type 2DM [13,14]. Adiponectin or adipocyte complement related protein of 30kd is an adipokine abundantly produced and secreted by adipose tissues [15]. It is a protein hormone of 244 amino acids that circulates in high concentration (5-30 micro gram /ml accounting, 1% of total serum proteins, its expression reduced in obese patients and pigs [16,17]. Adiponectin is encoded by the AMP1 gene (adipose most abundant gene transcript), on the long arm of chromosome 3. [18]. Adiponectin found in different complexes LMW trimer, MMW hexamer and HMW [12-18] mer, Since the total level of adiponectin but also the complex distribution may contribute to distinct down steam biologicaleffect [19]. The ratio of HMW to total level is amore reliable index for correlation with insulin sensitivity in both rodents and human studies.

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**Results:** The obtained results revealed significant (P<0.05) reduction in insulin and adiponectin (P<0.001) and elevation of blood glucose (P<0.001) in diabetic rats in group C, while significant (P<0.05) reductions in blood glucose, LDL levels and significant (P<0.05) elevation in adiponectin and HDL levels were detected in rats in group B and D.

**Conclusions:** The studies provide evidence that pearl millet induces hypoglycemic effect and improved lipemic control in diabetic rats.

**Keywords:** Diabetes mellitus; diet modifications; Wistar rats; pearl millet; lipids.
Type two diabetes mellitus may cluster with other conditions such as hypertension, obesity and hyperlipidaemia which increase the incidence of cardiovascular risk. It’s known that diet and lifestyle affect the incidence of diabetes. Diet plays an important role in controlling diabetes [20]. Eating wrong foods can accelerate diabetes, so dieticians can select appropriate intervention diet, [21] such options increase the likelihood of treatment to be successful. Problems with dietary intervention arise from different standards of different countries, the UK has different standards from therest of Europe [22]. The American diabetes association and the Canadian association are differering more differences can be observed in Japan, India and Africa, this mainly due to lifestyle differences, different cultures and dietary requirements in their local climate. Also, there are many variations in term of length of studies on dietary interventions of type 2DM [23]. Therefore, this experiment used Wistar rat model control and diabetes to compare the effect of Pearl millet on glycaemic and lipid control on days 0, 14 and 28.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty male healthy mature (Wistar) rats were used in the studies. The animals were kept in an animal house at the Department of Physiology. The rats divided into 4 groups, ten rats in each, and group (A) and (B) normal control rats while group (C) and (D) were considered as diabetic rats. The experiment 1, included two groups (A and C), equal rats and the parameters investigated were measured in days 0, 14, and 28. The experiment 2 included two groups (B and D) were received 20% pearl millet and the blood samples were measured in days 0, 14 and 28. Diabetes induced by intraperitoneal injection of 40 mg /kg streptozocin as a freshly prepared solution in 1 ml citrate buffer pH 4.5. Rats were provided 5% glucose solution in place of water to prevent drug induced hypoglycemic mortality. At the third day after injection blood was drawn from the retro orbital artery of overnight fasting rats and used to assess their hyperglycemic status by measuring of blood glucose levels. Rats with fasting blood glucose more than 250 mg/dl was considered to be diabetic.

2.2 Experimental Procedure

The complete randomized design was used to evaluate the effect of Pearl millet on glycaemic control and lipid profile in streptozocin induced diabetic rats. Groups B control and D diabetic were feeding pearl millet by mouth by using syringe calculated according to the bodyweight during experimental periods. Blood was withdrawn from retro orbital artery by heparinized capillary tube form one eye for sampling in days 0, 14 and day 28 to determine blood glucose and lipid profile.

2.3 Extraction of Pearl Millet

The percolation cold method of extraction was applied. A 500 mg of pearl millet was dried under the shed and powdered. Then the powder was packed inside the percolator apparatuses. A suitable amount of ethanol 80% was added inside the percolator until all the millet powder material emerged by the solvent. The percolator was tightly closed and kept for 2 days. The ethanol extract was obtained and then another quantity of ethanol 80% was added, the percolator was kept for 2 days. This procedure was repeated 3 times after that the collected ethanol was extracted and evaporated under reduced pressure. Lastly the extracted and weighted (to calculate the yield percentage) and stored, the closed bottle was kept at 4°C until it is used for biological assay.

2.4 Collection of Blood Samples

The rats were made comfortable and restrained and the area of collection scrubbed by disinfectant (70% ethanol) and topical local anaesthetic Lidocaine 1% was applied to the eye before sampling. Then 2 ml of blood was collected in aplastic container tube by using a standard heparinized capillary tube was placed in the medial canthus of the eye under nictating membrane and blood withdrawn into the collecting tube and slight pressure with a piece of gauze was applied to prevent further bleeding and restrainer was washed to avoid infection.

2.5 Blood Glucose Measurement

Fasting blood glucose was measured on days 0, 14 and 28 by enzymatic colorimetric method [24] using commercial kits (Biodiagnostic). The intensity of the color is proportional to the glucose in the sample. The colorimetric determination was performed in a spectrophotometer.

2.6 Low Density Lipoprotein (LDL)

The LDL cholesterol was determined by enzymatic colorimetric method by using LDL rat
kits (SinoGene Clon Coltd). The method described by Wieland and Seidel [25].

2.7 High Density Lipoprotein (HDL)

The HDL cholesterol was determined by enzymatic colorimetric method by using HDL rat kits (SinoGene Clon Coltd). The method described by Burstein et al. [26].

2.8 Insulin Concentration

Insulin was measured by using Rat insulin ELISA kits (SinoGen Clon ltd) method sandwich ELISA detection (Asys Exert Plus, Austria).

2.9 Adiponectin Concentration

Adiponectin was measured by using Rat Adiponectin ELISA kits (Sino Gene Clon ltd) by method sandwich ELISA detection (Asys Exert Plus, Austria).

2.10 Statistical Analysis

The data obtained from the studies were subjected to standard methods of statistical analysis using the Statistical Package of Science and Social (SPSS) version 16.0. The experiments were performed according to the complete randomized design (CRD). Analysis of variance (ANOVA) test was used to evaluate the effect of Pearl millet on glycaemic control and lipid profile in streptozocin induced diabetic rats. The mean values were compared for significance at P≤0.05 or P≤0.001 and group of results were presented as mean ± SD.

3. RESULTS

The serum biochemical parameters in control and streptozocin diabetic rats presented in Table 1.

3.1 Insulin (µ/ml)

The results show that, the gradually significant (P≤0.05) reduction of insulin level was detected in diabetic rats on day 28 compared to the control values have shown in Table 1.

3.2 Adiponectin (µ/ml)

The current results confirmed that the adiponectin concentration was significantly (P ≤ 0.001) decreased in control rats and increased in the diabetic group during experimental periods and minimum concentration was detected in diabetic rats in day 0 in Table 1.

3.3 Blood Glucose (mg/dl)

The present study shows that, highly significant (P≤0.001) increased of blood glucose levels in diabetic rats compared to the control group in days 0, 14 and 28. Highest level of blood glucose documented in day 0 in diabetic rats.

3.4 Low Density Lipoprotein (mg/dL)

The results of this study confirmed that, the LDL levels significantly (P≤0.05) increased in diabetic rats and decreased in control groups throughout experimental periods shown Table 1.

3.5 High Density Lipoprotein (mg/dL)

The current study showed significant (P≤0.05) increased of HDL concentrations in both groups in Day 0 and 14 shown in Table 1.

3.6 Experiment 2

The effect of pearl millet in blood glucose level, adiponectin, lipid profile and insulin levels were presented in Table 2.

3.6.1 Insulin (µ/ml)

This study revealed that, there were no significant changes in insulin level were detected in the control and diabetic rats treated by Pearl millet throughout the experimental period presented in Table 2. But the general trend indicates an numerical increase in insulin level in diabetic rats fed pearl millet.

3.6.2 Adiponectin (µ/ml)

The results revealed significant (P≤0.05) increased of adiponectin concentrations in diabetic rats compared to the control group fed pearl millet in days 0, 14 and 28 shown in Table 2.

3.6.3 Blood glucose (mg/dl)

This study reveals that, significant (P≤0.001) decreased in blood glucose levels in both groups fed pearl millet in days 0, 14 and 28. The reduction was more prominent in diabetic rats shown in Table 2.
Table 1. Serum biochemical parameters in control and streptozocin diabetic rats (Groups A and C) in days 0, 14 and 28

<table>
<thead>
<tr>
<th>Days</th>
<th>Insulin (μ/l)</th>
<th>Adiponectin (μ/l)</th>
<th>Blood glucose (mg/dL)</th>
<th>Low density lipoprotein (mg/dL)</th>
<th>High density lipoprotein (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
</tr>
<tr>
<td>0</td>
<td>3.89±1.07</td>
<td>3.51±0.37</td>
<td>41.67±20.66</td>
<td>4.49±4.05</td>
<td>71.80±14.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59.50±9.76</td>
<td>58.75±9.32</td>
<td>38.70±9.67</td>
</tr>
<tr>
<td>14</td>
<td>3.12±0.60</td>
<td>2.75±0.93</td>
<td>38.70±16.97</td>
<td>5.93±4.28</td>
<td>80.10±13.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>57.50±8.07</td>
<td>49.25±5.75</td>
<td>39.20±12.43</td>
</tr>
<tr>
<td>28</td>
<td>3.21±0.57</td>
<td>2.10±0.64</td>
<td>14.30±7.18</td>
<td>12.00±10.65</td>
<td>76.00±12.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59.20±11.94</td>
<td>55.29±4.16</td>
<td>41.50±8.10</td>
</tr>
<tr>
<td>P-value</td>
<td>0.049</td>
<td>0.001</td>
<td>0.001</td>
<td>0.026</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Mean ± SD values, letters bearing different superscripts are significantly different at (P≤0.05) or highly significant at (P≤0.001) and which have the same superscripts are non-significant.
3.6.4 High density lipoprotein (mg/dl)

The results of this study reveal that, significant (P≤0.05) increased of HDL concentrations in diabetic rats and control group fed pearl millet in days 0, 14 and day 28 have shown in Table 2.

3.6.5 Low density lipoprotein (mg/dl)

The results revealed significant (P≤0.05) decreased of LDL concentrations in diabetic and control rats fed pearl millet in days 0, 14 and 28 shown in Table 2.

Fig. 1. Insulin in control and streptozocin diabetic rats in days 0, 14 and 28

Fig. 2. ADP in control and streptozocin diabetic rats in days 0, 14 and 28

Fig. 3. Blood glucose in control and streptozocin diabetic rats in days 0, 14 and 28
Fig. 4. Low density lipoprotein in control and streptozocin diabetic rats in days 0, 14 and 28

Fig. 5. High density lipoprotein in control and streptozocin diabetic rats in days 0, 14 and 28

Fig. 6. Effect of pearl millet in insulin in control and streptozocin diabetic rats in days 0, 14 and 28
Table 2. Effect of pearl millet in serum biochemical parameters in control and streptozocin diabetic rats (groups B and D) in days 0, 14 and 28

<table>
<thead>
<tr>
<th>Days</th>
<th>Insulin (µ/ml)</th>
<th>Adiponectin (µ/ml)</th>
<th>Blood glucose (mg/dL)</th>
<th>High density lipoprotein (mg/dL)</th>
<th>Low density lipoprotein (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
</tr>
<tr>
<td>0</td>
<td>1.00±0.15</td>
<td>0.97±0.19</td>
<td>8.50±1.87</td>
<td>5.97±3.60</td>
<td>155.17±66.26</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.83±1.17</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Control rats</td>
</tr>
<tr>
<td></td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
</tr>
<tr>
<td>14</td>
<td>1.00±0.08</td>
<td>1.03±0.04</td>
<td>6.83±1.17</td>
<td>13.25±3.20</td>
<td>74.37±9.37</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>12.50±1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control rats</td>
</tr>
<tr>
<td></td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
<td>Diabetic rats</td>
<td>Control rats</td>
</tr>
<tr>
<td>28</td>
<td>1.03±0.05</td>
<td>1.04±0.04</td>
<td>12.88±2.64</td>
<td>16.00±4.15</td>
<td>64.33±14.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.00±2.61</td>
</tr>
<tr>
<td></td>
<td>P-value &gt;0.05</td>
<td></td>
<td></td>
<td></td>
<td>0.048</td>
</tr>
</tbody>
</table>

Mean±SD values; letters bearing different superscripts are significantly different at (P≤0.05) or highly significant at (P≤0.001) and which have the same superscripts are non-significant.
Fig. 7. Effect of pearl millet in ADP in control and streptozocin diabetic rats in days 0, 14 and 28

Fig. 8. Effect of pearl millet in blood glucose in control and streptozocin diabetic rats in days 0, 14 and 28

Fig. 9. Effect of pearl millet in high density lipoprotein in control and streptozocin diabetic rats in days 0, 14 and 28
4. DISCUSSION

Diabetes mellitus is a syndrome of chronic hyperglycemia due to absolute or relative insulin deficiency. Millet is effective in improving lipid metabolism and glycaemic control. This study was designed to investigate the effect of Pearl millet on glycaemic control and lipid profile in the streptozocin diabetic rat model on days 0, 14 and 28.

This study revealed that, no significant changes in insulin level detected in the control and diabetic rats fed Pearl millet throughout the experimental periods. But the general trend indicates numerical increase of insulin level in diabetic rats fed millet was detected on days 14 and 28. These results are in agreement with the results of Nishawa et al. who reported that, millet increased insulin sensitivity and reduced blood glucose and triglyceride levels in diabetic mice [27]. Recently Trembley et al. found that dietary protein and amino acids can modulate glycaemic control and insulin secretion [28]. However, Marz et al. reported that, reductions in insulin, lipid, Hba1c and fasting glucose have a positive implications on cardiac health [29].

The results of this experiment showed significant (P ≤ 0.05) elevation of adiponectin associated with highly significant (P ≤ 0.001) reduction in blood glucose levels throughout the study periods. The findings imply that, feeding with such diet can play an important role to restore the plasma level of adiponectin to the physiological level. It is well established that increase adiponectin level stimulate glucose utilization through activation of AMP activated protein kinase in skeletal muscle and liver [30] such a diet contain pearl millet could reduce glucose level due to enhancement of utilization of glucose by peripheral tissues and elevates adiponectin level. Many theories supporting the hypoglycemetic effects of pearl millet such as Pearl millet is rich in phytate and phenolic compounds that reduce fasting hyperglycemia and attenuated postprandial blood glucose response in rats [31]. Phenolic compounds are also known to enhance insulin activity was reported by Anderson and Polansky [32] also, millet regulates intestinal GLUT, increases muscle glucose uptake and reduces hepatic gluconeogenesis [33]. Our findings are similar to results of Hedg et al. [34] which reported 36% reduction in blood glucose level in alloxan induced diabetic rats fed with millet and Shanmugan et al. [35] who reported 39% reduction in glucose level in rats fed with finger millet. Also similar to findings of Nishawa et al. [27] which documented reduction in blood glucose in rats fed with Japanese millet and comparative study of Jali et al. [36] which reported 13% reduction in glucose level in humans fed with foxtail millet.

Hypercholesterolemia and hypertriglyceridemia are the usual complications of diabetes with their serious consequence such as atherosclerosis. In this study, the diabetic rats fed with millet exhibited significant (P ≤ 0.05) elevation in HDL and significant (P ≤ 0.05) reduction in LDL level. The mechanism by which dietary pearl millet affects HDL and other lipid profile levels is unclear. Some theories suggesting that, the elevation of lipid profile could be related to high prolamin fraction in pearl millet. The prolamin fraction prepared from Japanese millet decrease blood glucose and insulin and elevated gene expression of adiponectin and down regulation of gene expression of TNF which is
already known that increase gene expression of TNF reduce gene expression of plasma level adiponectin and some previous studies mentioned suggest that different dietary proteins differentially modulate metabolism [37] and dietary proteins are abetter choice and more effective way to modulate glycaemic response and lipid profile, insulin and adiponectin levels.

5. CONCLUSION
The studies concluded that, pearl millet induces potential hypoglycemic effect and improved lipidemic control in the diabetic rat model and further studies were advised.

CONSENT
It is not applicable.

ETHICAL APPROVAL
The ethical issues were addressed adequately according to veterinary and institutional guidelines.

DISCLAIMER
The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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