Anti-Hyperglycemic Effects of Okara, Corn Hull and Their Combination in Alloxan Induced Diabetic Rats

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Abstract: Okara and corn hull are both considered waste by-products that are rich in dietary fiber. The present study was carried on to investigate the hypoglycemic effects of okara, corn hull and their combination in diabetic rats. Diabetes was induced in male Sprague-Dawley rats by intraperitoneal injection of 120 mg/kg alloxan monohydrate in saline. Diabetic rats were then randomly divided into five groups and received either normal diet (positive control) or diet supplemented with 10% of okara, corn hull individually and mixture of them (50:50) for 4 weeks. Chemical composition and total phenolic content of both okara and corn hull were estimated. The studied parameters included fasting blood glucose, serum insulin and glycated hemoglobin levels, changes in body weights, feed intake and the histopathological changes in the spleen. Results showed that okara contained significantly higher protein and fat than that of corn hull. Total dietary fiber and polyphenols values were found to be significantly greater in corn hull than in okara. Diabetic rats fed diets supplemented with either okara or corn hull individually or a mixture of both gained less weight than that of the diabetic control group. Compared with positive control group, all treatments caused a significant reduction in fasting serum glucose levels, glycated hemoglobin and significant increase in serum insulin. The effect was more pronounced in the okara + corn hull supplemented group. Supplementation of either okara or corn hull improve to some extent the histopathological changes observed in the pancreas compared with positive control group. Significant islet structure restoration was observed in diabetic rats on okara+ corn hull supplemented diet as compared with the positive control group. Our findings provided evidence that the combination of okara and corn hull in the diet of diabetic patients might be of great beneficial effects in glycemic control and in reducing the risk of diabetic complications. Further research is required to determine the other health and nutritional benefits of these by-products.

Keywords: Okara · Corn hull · Diabetic rats · Chemical composition · Insulin · Fasting glucose · Glycosylated hemoglobin · Spleen histology

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin [1]. Diabetes mellitus is currently one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world [2]. The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to be more than double by 2030 [3]. The highest prevalence of diabetes as well as its long-term complications has led to an ongoing search for hypoglycemic agents from natural sources [4]. A high dietary fiber intake is emphasized in the recommendations of most diabetes and nutritional associations [5]. The beneficial metabolic effects of dietary fiber are longlasting and clinically relevant both in type 1 and type 2 diabetic patients [6-8].

By-products of plant food processing represent an important disposal problem for the concerning industry, but they are also promising sources of compounds which can be used because of their favorable technological or nutritional properties and today they are considered as a possible source of functional compounds [9]. Dietary fiber
has all the characteristics required to be considered as an important ingredient in the formulation of functional foods [10]. The health importance of food fiber has led to the development of a large potential market for fiber rich products and ingredients. Nowadays, there is a trend to find new sources of dietary fiber, such as agronomic by-products that have traditionally been undervalued [11].

Okara is a by-product of the soy milk and tofu industry. Raw okara, also called soy pulp, which is a white-yellow material consisting of insoluble parts of the soybean seed remaining in the filter sack when pureed soybean seeds are filtered in the production of soy milk. This by-product has been used in the vegetarian diets of Western countries since the 20th century. At least (1.1 kg) of fresh okara is produced from every kilogram of soybeans for soy milk production. It contains mostly crude fiber (50-60%); composed of cellulose, hemicellulose and lignin; protein, oil, but little starch or simple carbohydrates [12]. Corn hull (hull of corn kernel) is obtained as by-product during the corn starch manufacturing process from dent corn. A large quantity of corn hull is produced in the world every year and they are mostly used for livestock feed. Corn hull contains much water insoluble dietary fiber and some refined corn dietary fiber products are marketed by several companies [13].

Therefore, the present study was designed to evaluate the anti-hyperglycemic effects of each of the two fiber sources (okara, corn hull) and their combination in alloxan-induced diabetic rats.

**MATERIALS AND METHODS**

**Materials:**

- Okara was provided as a fresh by-product from soybean (*Glycine max* L.) by Scybean Research Center, Agriculture Research Center, Cairo, Egypt.
- Corn hull was obtained from South-Cairo Milling Company, El-Tiban, Helwan, Egypt.
- Alloxan was obtained from El-Gomhuryia Company for Chemical Industries, Cairo, Egypt. All diagnostic kits were purchased from Gamma Trade for Scientific Services and Consultation, Giza, Egypt.
- Thirty male Sprague Dawley rats weighing about 120–140 g were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA) Cairo, Egypt.

**Methods:**

**Chemical Composition:** Moisture, protein, fat and ash in okara and corn hull samples were determined by official methods of analysis [14]. Moisture content of okara was determined by weight loss after oven drying to a constant weight at 105°C. Total nitrogen was determined using the micro-Kjeldahl method. Fat content was determined by extraction with petroleum ether using the Soxhlet method. Ash content was measured as the residue obtained after incineration at 550°C for 3 h. Dietary fiber was analyzed using enzymatic–gravimetric method, fractionating into insoluble and soluble residues based on the method published by AOAC [14]. Available carbohydrates were calculated from mean values by difference. Also, total polyphenols was determined by the Folin–Ciocalteu procedure using gallic acid (GA) as standard [15]. All determinations were performed four times and were reported on a dry matter basis.

**Experimental Induction of Diabetes:** Rats were housed individually in cages under controlled conditions. They were maintained on a basal diet (AIN-93) [16] for 1 week as adaptation period. All diets and water were provided *ad libitum*. Rats were injected intraperitoneally with a freshly prepared solution of alloxan monohydrate in saline (300mM NaCl) at a dose of 120mg/kg of bodyweight after overnight fasting for 12 h [17, 18]. Since alloxan injection can provoke fatal hypoglycemia as a result of reactive massive release of pancreatic insulin, rats were kept for the next 24 h on a 5% glucose solution as beverage to prevent severe hypoglycemia [19]. Fasting blood samples were collected from the retroorbital sinus in anesthetized rats using a microhematocrit tube and blood glucose levels were measured to investigate the induction of diabetes. Rats displaying blood glucose level of 250 mg/dl were defined as diabetics and were chosen for the experiment [20-22].

**Experimental Design:** The experimental animals were divided into five groups (six rats per group): (1) normal control group (designated NC) fed basal diet, (2) Alloxan induced diabetic control group (DM) fed basal diet, (3) DM rats fed basal diet supplemented with 10% okara (DM-okara), (4) DM rats fed basal diet supplemented with 10% corn hull (DM-corn hull) and (5) DM rats fed basal diet supplemented with 5% okara + 5% corn hull (DM-okara + corn hull) for 4 weeks.
Measurement of Body Weight and Food Intake:
Body weight and food intake were measured every day at the same hour during the experimental periods. The FER was calculated as daily weight gain (g)/daily dietary intake (g).

Biochemical Analysis: Animals were lightly anesthetized with diethyl ether after 12 hours of fasting at the end of the experimental period and blood was collected from the retroorbital sinus. Blood samples were centrifuged and sera were obtained.

- Blood glucose was estimated by commercially available glucose kit based on glucose oxidase method [23].
- Serum insulin concentration was measured by using an immunoradiometric assay kit [24].
- Glycosylated hemoglobin (HbA1c) was determined using a commercial kit [25].

Histological Evaluation: After the animals were sacrificed, small pieces of the pancreas were taken from the experimental animals and were fixed in 10% neutral formalin, alcohol-dehydrated, paraffin-embedded and sectioned to a mean thickness of 4 mm. The sections were stained with hematoxylin and eosin before being observed under a light microscope for histological examination [26].

Statistical Analysis: The results were expressed as (mean ± SD) values. Statistical analysis of differences between the chemical composition of okara and corn hull was performed using student's t test. Statistical analysis of differences between the different groups was carried out using one-way analysis of variance test followed by Duncan's post hoc test (SPSS statistical software package, version 16.0, SPSS, Chicago, IL). A value of (P < 0.05) was accepted as the level of statistical difference.

RESULTS AND DISCUSSION

Okara and corn hull are by-products of food industries which have not been extensively studied, especially as a potential source of functional components. Data presented in Table 1 showed that okara contained significantly (P < 0.05) higher protein and fat than that of corn hull. No differences were found with regard to carbohydrate content between the two by-products.

<table>
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<tr>
<th>Table 1: Chemical Composition and Polyphenols Content of Okara and Corn Hull (g/100 g dry weight)</th>
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Fig. 1: Fiber Content in Okara and Corn Hull

Total polyphenols values were found to be significantly (P < 0.05) greater in corn hull than in okara. The okara polyphenols value obtained in the present study agreed with that reported by other study in which stated that the estimated total mass of polyphenols (isoflavones) lost in the okara was 220 mg / 100 gm. There was 84 mg daidzein, 130mg genistein and 4.0 mg glycitein in 418 g okara [27]. Total dietary fiber values (Fig. 1) were found to be significantly (P < 0.05) greater in corn hull than in okara representing 88.81 ± 3.52 vs. 55.63 ± 1.48 g / 100 g dry weight respectively. The trend was observed regarding soluble and insoluble fiber. It consisted mainly of soluble fibers. On the other hand, Redondo-Cuenca et al. [28], Espinosa-Martos and Ruperez [29] and Jimenez-Escrig et al. [30] reported differences to some extent in okara chemical composition than what found in the present study. This could be attributed to the variety of soybean, geographical locations or the processing factor. However, the present results concerning chemical composition of corn hull were considered to be within the range reported by Sugawara et al. [13].

The present study showed that alloxan treatment produced a significant (p < 0.01) reduction in body weight of diabetic control rats, whereas the normal control group rats continued to gain weight as reported in Table 2.
Diabetic rats fed diets supplemented with either okara or corn hull individually or a mixture of both gained less weight than that of the normal control group but markedly higher than that of the diabetic control group. The lowest increment was observed in corn hull supplemented group while the highest increment was observed in okara supplemented group. Similar to the results of weight gains, FER values in all alloxan treated groups were significantly \( (p < 0.01) \) lower than that of the normal control group. However, FER of the diabetic group supplemented with okara was higher than that of the diabetic control group. Alloxan injection also produced a significant \( (p < 0.01) \) increase in daily feed intake (from \( 10.39 \pm 0.46 \) to \( 14.56 \pm 0.92 \) g/day) for normal and positive control group, respectively \( (p < 0.05) \). Treatment of diabetic rats with okara and mixture of okara and corn significantly \( (p < 0.01) \) decreased the daily feed intake compared with normal rats.

It is well known that markers of diabetes, in both humans and animal models, include limited weight gain, polyuria, polydipsia and polyphasia, which are direct consequences of insulin insufficiency [31]. Therefore, body weight gains and feed intake serve as indicators of proper glucose handling. These results suggested that all supplements especially okara regulate the various symptoms of diabetes, such as growth retardation and polyphasia. In agreement with the present results, Yadav et al. [32] and Song et al. [33] found that the hyperglycemia induced by alloxan was associated with less in body weight resulting from increased muscle wasting [34] and due to loss of tissue proteins [35].

On the light of this, supplementation with okara which contained higher amount of protein than corn hull have a protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis and in proper glycemic control.

As expected, serum glucose levels were significantly increased in the alloxan induced diabetic rats compared with the negative control rats (Table 3). Compared with positive control group, all treatments caused a significant \( (p < 0.05) \) reduction in serum glucose levels. However, compared with okara or corn hull supplemented groups, serum glucose levels were significantly \( (p < 0.05) \) lower in the okara + corn hull supplemented group (136.75 ± 10.26 mg/dl) compared with positive control group (145.25 ± 9.74 mg/dl). On the other hand, serum insulin levels were lower in the alloxan induced diabetic rats compared with the negative control rats \( (p < 0.05) \). The supplementation of all treatments increased the serum insulin level of the alloxan induced diabetic rats. The effect was more pronounced \( (P < 0.05) \) in the okara + corn hull supplemented group compared with positive control group \((10.46 \pm 0.88 \text{ ng/dl} \text{ vs. } 4.31 \pm 0.41 \text{ ng/dl})\).

It is possible that several factors and their interactions played a role in the observed effects. One possible mechanism by which all supplements bring their hypoglycemic action is attributed to the high fiber content of both okara and corn hull supplements. In accordance with the present study, it was reported that an increase in the intake of total dietary fiber, especially from soluble fiber, significantly improved glycemic control in diabetic patients especially type II diabetes [36].
Possible mechanisms for metabolic improvements with dietary fiber include delay of glucose absorption from intestine, increase in pancreatic extraction of insulin and increased insulin sensitivity at the cellular level [6, 37-39].

In human, reactive oxygen species play critical roles in the pathogenesis of diabetes and the development of diabetic complications [40, 41]. In animal model, alloxan produces selective cytotoxicity in pancreatic β-cells of the islets of Langerhans through the generation of reactive oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation and resulting in reduced synthesis and release of insulin [42, 43]. The present study showed an increase in serum insulin levels in diabetic rats fed on diets supplemented with okara, corn hull or mixture of both compared with those levels found in rats in the positive control group. As a result of this, increased insulin secretion improves serum glucose control. These findings suggested that supplementation with okara; corn hull and their combination protect residual β-cells against glucose toxicity or toxic effects of alloxan and improves β-cell function (that was coincided with the histopathological studies of the pancreas). The prevention of the functional β-cells of the Langerhans islet from further deterioration enhances insulin secretion. The presence of biologically active constituents in both okara and corn hull is one possible mechanism responsible for the potent improvement in serum insulin level.

Loss of soybean isoflavones through the byproducts, okara was considerable [27] and it is well known that isoflavones have been credited with performing several health-promoting functions. Studies have demonstrated that soy protein isoflavones confer a potential anti-diabetic effect in animals and humans [44, 45]. Among the isoflavones that were found in okara is genistein. Genistein supplementation increase level of serum insulin and have hypoglycemic effect on STZ-diabetic rats [46] through its effect on stimulating insulin secretion of the β-cells [47]. Similarly, Striffier and Nadler [48] and Lu et al.[49] reported that the beneficial effects of soy isoflavones were attributed to increased insulin secretion, better glycemic control and antioxidants protection. However, because the soy product "okara" contained both the protein and isoflavones, there is no way to determine if this effect was due specifically to isoflavones. So okara protein is another partner in the observed effect. Beside the role of protein in maintaining body composition and nitrogen balance, protein is a potent stimulant of insulin secretion as glucose without contributing to sustained elevation of glucose levels [50].

Fig. 2: Mean Glycosylated Hemoglobin Level in Control, Diabetic Rats and Treated Diabetic Rats.

Corn hull contain phenolic compounds. Zhao et al. [51] stated that consumption of corn hull but not wheat hull significantly increased the concentration of total phenolic antioxidant such as ferulic acid in plasma. Phenolic compounds have been found to be beneficial in controlling diabetes and many other diseases as evident from several studies [52- 54]. In addition to phenolic compounds, corn hull has a high L-arabinose content and its functional potential is highly attractive [55]. L-arabinose inhibits intestinal sucrase activity and thus suppresses increasing plasma glucose [56] and is therefore expected to be important for diabetic therapy. Hence, the beneficial effect of corn hull supplementation resulted from the presence of these compounds in addition to its fiber content. Potentiating the insulin effects of serum by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form are also considerable factors.

Glycosylated hemoglobin (HbA1c) levels were significantly higher ($P < 0.05$) in the alloxan induced diabetic groups compared to the negative control group (Fig. 2). This increase is directly proportional to the fasting blood glucose levels [57]. Since the diabetic animals had prior higher blood glucose levels, elevated levels of HbA1c were observed. All treatments decreased the HbA1c level of the diabetic rats compared to positive control group ($P < 0.05$). The effects were more potent in the okara + corn hull group than in the other treated groups. This effect seems to be due to an improvement in insulin secretion and the reduction of blood glucose level. In the light of our results, it was reported that the beneficial metabolic effects of dietary
fiber are long-lasting and clinically relevant, as shown by the lower plasma glycosylated hemoglobin levels both in type 1 and type 2 diabetic patients [8].

The histopathological observations of pancreatic tissue in the normal and diabetic experimental groups were presented in Fig. 3. No histopathological changes were observed in the pancreas of negative control group (Fig. 3-A). The structure of the islets of Langerhans was highly destroyed after alloxan injection (Fig. 3-B). Few typical islets of Langerhans could be identified and many β-cells showed vacuolations. Supplementation of either okara or corn hull improve to some extent the histopathological changes observed in the pancreas of positive control group. Pancreas of rats from okara supplemented group showed slight vacuolations of β-cells of islets of Langerhans (Fig. 3-C), while pancreas of rats from corn hull supplemented group showed slight hyperplasia and hypertrophy of islets of Langerhans (Fig. 3-D). Significant islet structure restoration was observed in diabetic rats fed on okara+ corn hull supplemented diet (Fig. 3-E), as compared with the positive control group. These results suggested that the serum level of insulin and histological findings provide strong evidence for cytoprotective effects of okara and corn hull supplementation.

In conclusion, our findings provided evidence that the combination of okara and corn hull in the diet of diabetic patients might be of great beneficial effects in glycemic control and in reducing the risk of diabetic complications. Further research is required to determine the other health and nutritional benefit of these by-products.

REFERENCES


