Hypoglycemic and Hypolipidemic Effect of Gamma-Irradiated Corn Silk on Male Albino Rats.

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ABSTRACT

Diabetes mellitus (DM) is a common and serious metabolic disorder throughout the world. Several herbs have been known to cure and control diabetes without any side effects. Corn silk (CS) is one of these herbs that are rich in phenolic and flavonoid compounds that have antioxidant activity. The purpose of this study is to investigate the hypoglycemic and hypolipidemic effect of gamma-irradiated corn silk on male albino rats. In this study, γ-radiation processing resulted in increase the total phenolic contents and the total antioxidant activity of raw CS. The results of this work showed that dietary supplementation with raw and γ-irradiated corn silk to alloxan-induced diabetic rats resulted in an obvious enhancement in the damage effects induced by alloxan by increasing the level of insulin, high density lipoprotein (HDL-C), glutathione content and the activity of SOD and CAT. Also, a remarkable decrease was observed in the level of glucose and malondialdehyde and the concentration of total cholesterol (TC), triglycerides (TG), low density- and very Low density lipoprotein-cholesterol in addition to the activity of some liver enzymes as a result of treatment of alloxan-induced diabetic rats with raw and γ-irradiated corn silk. In conclusion, the results revealed that either raw or γ-irradiated corn silk reduced hyperglycemia in alloxan-induced diabetic rats may be used as a hypoglycemic food for diabetic patients. Further, this study indicated that treatment of CS with 10 kGy of gamma irradiation raised the total phenolic contents without any significant effect on its properties.

INTRODUCTION

Diabetes is a serious endocrine and metabolic disorder caused by a combination of resistance to insulin action. Among the diabetic cases, Type 2 diabetes accounts for more than 90% (1&2). Hyperlipidemia, elevated levels of lipids in the blood plasma, is a condition commonly observed in patients with type 2 diabetes. Diabetes and hyperlipidemia are two major factors involved in the development of cardiovascular disease (3&4). The medical treatment and reduction of the effects of these conditions are key modalities in the prevention of heart disease (5).

Corn silk, an outer thread-like part of corn, is made up of the stigmas and styles of the maize plant belonging to the grass (Gramineae) family (6). It has been found that corn silk is an excellent source of many bioactive compounds such as flavonoids, saponin, alkaloids, chlorogenic acid, phytosterols, vitamin E and K and also it contains different minerals such as calcium, magnesium and iron (7&8). Flavonoids from corn silk (FCS) have been investigated and confirmed to possess various pharmacological activities such as antihypertensive, anti-infectious, anti-oxidative and anti-diabetic (9&10). Therefore, corn silk can be used in the manufacture of health foods and treatment of different diseases such as diabetes (11).
Herbs are often contaminated with microorganisms from the plants themselves, soil, water, air and dust during post-harvest and processing, and it could be a cause of serious food-borne illness (12). Irradiation is a physical process in which high-energy ionizing radiation passes through food and improves food safety by the inactivation of microorganisms. In 1981, the JECFI (FAO/IAEA/WHO Joint Expert Committee on the Wholesomeness of Food Irradiation) stated that “the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazards” and “introduces no special nutritional or microbiological problems” (13).

The present study aimed to examine the hypoglycemic and hypolipidemic effect of gamma-irradiated corn silk in male diabetic albino rats.

MATERIAL AND METHODS

Material:
Alloxan monohydrate was purchased from Sigma Chemical Company. Fresh corn silk was collected from the farm at Qalubia (Egypt) as agricultural residues. Then it was washed with distilled water, and dried for 24 h by using a hot air oven at 60°C. The dried corn silk was ground into a powder form, sieved and kept in an airtight container at 4°C until used.

Irradiation process:
Powder of corn silk transferred into polyethylene bags and treated with 10 kGy of gamma rays, using a 60Co source at a dose rate of 4.70 KGy/h at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

Determination of total phenolic compounds:
The water extract of raw and γ-irradiated corn silk was prepared by adding 10 g of dried corn silk into 100 mL of hot water for 15 min with constant stirring and was vacuumed-filter twice, centrifuged (3,000 rpm, 10 min).

The total phenolic contents of raw and γ-irradiated corn silk water extract were determined by using the Folin-Ciocalteau calorimetric method (14). Briefly, 1 mL of sample was added into a 25 mL volumetric flask followed by the addition of 1 mL Folin-Ciocalteau reagent (1N). The mixture was shaken slowly and left to react at room temperature for 5 min. After 5 min, 10 mL of sodium bicarbonate (7% w/v) was added into the mixture. The flask was filled with distilled water and left to stand at room temperature in the dark for 40 min. Distilled water was used as blank. Sample absorbance was recorded at 750 nm against the blank. Sample absorbance was compared to that of gallic acid standard curve previously prepared covering the concentration of 20 to 100 μg/mL. Samples were measured in triplicate analysis.

Determination of Antioxidant Activity by the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH):
DPPH assay was determined by a specific method described (15). Each sample (0.5 mL) was added to 0.5mL of 0.4 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand for 30min; the absorbance of the resulting solution was measured at 517nm with a spectrophotometer. Percent inhibition of DPPH radical was calculated for each dilution of CS extract according to formula: %inhibition = ((ADPPH−ACS) /ADPPH)) × 100, where ADPPH is the absorbance value of the DPPH versus blank solution and ACS is absorbance value of the sample solution. A lower level of absorbance indicated a stronger radical scavenging activity.

Study design:
Male albino rats weighing approximately 180-200g were used in this study. Hyperglycemia was induced by intraperitoneal administration of alloxan monohydrate dissolved in saline at a dose of
150 mg/kg body weight (16). Alloxan can be induced fatal hypoglycemia as a result of massive pancreatic insulin release; therefore, rats were treated with 30% glucose solution orally after 6 h of alloxan induction, and 5% glucose solution was kept in bottles in their cages for the next 24 h. After one week, blood was extracted from the tail vein for glucose analysis by the method of Trinder (17). Experimental animals exhibited fasting blood glucose levels in the range of 200 to 250 mg/dl.

Experimental Animals:

Adult male albino rats, reared in the NCRRT, Egypt, were used in the present work. Rats were housed in plastic cages under controlled condition and fed on well balanced diet (20% casein, 10% soybean oil, 5% cellulose, 4% mineral mix, 2% vitamin mix, 6% sucrose, and 53% cornstarch). The experimental diet was prepared according to the formula recommended by the AIN-76 (18) guidelines. The animal were randomly divided into 4 groups, each consisted of 7 rats, as follows: Group C: rats fed on balanced diet and served as control, group D: (diabetic group) rats fed on balanced diet for 8 weeks and administrated with alloxan (150 mg/kg B.wt.) after the 2nd week of the experimental period. Groups D+CS & D+Irr. CS: rats fed on balanced diet supplemented with 1% of either raw or γ-irradiated corn silk powder for 8 weeks and administrated with alloxan (150 mg/kg B.wt.) after the 2nd week of the experimental period.

At the end of the experimental period, the rats in each group were fasted overnight, anaesthetized with diethyl ether and sacrificed. Blood samples were collected through heart puncture, allowed to coagulate and centrifuged to obtain serum for biochemical analysis. Also, liver was removed and prepared for biochemical investigation.

Biochemical Analysis:

Serum glucose level was measured by the method of Trinder (17) and also insulin hormone level was determined by radioimmunoassay kit supplied by Diasari, Italy. The lipid peroxidation products were determined colorimetrically as malondialdehyde (MDA) according to Yoshioka et al. (19). Whereas, the value of hepatic glutathione content (GSH) and the activity of superoxides dismutase (SOD) and catalase (CAT) were measured by the method of Gross et al. (20), Minami and Yoshikawa (21) and Aebi (22), respectively. In addition, total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain et al. (23), Fossati and Prencipe (24) and Demacker et al. (25), respectively, while low-density lipoprotein cholesterol and very Low-density lipoprotein-cholesterol were evaluated according to Friedwald et al. (26) and Norbert (27) formulas, respectively by the following equations: LDL-C (mg/dl) = TC - (TG/5+HDL-C), vLDL (mg/dl) = TG/5. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (28), serum gamma glutamyl transferase (GGT) was assessed according to Rosalk (29), and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (30).

Statistical analysis:

Results were presented as mean ± SE (n = 7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan’s multiple range test was used to determine significant differences between means. Statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS) (31). Differences between means were considered significant at P < 0.05.
RESULTS

The amount of total phenolic compounds and the total antioxidant activity of CS are shown in Table (1); the results showed that the total phenolic contents of raw and $\gamma$-irradiated CS were 119.72 and 126.35 mg GAE/g dry CS, respectively. While the total antioxidant activity was 84.23 and 89.46 mg/mL, respectively.

Table 1: The total phenolic contents and total antioxidant activity of raw and $\gamma$-irr. CS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CS</th>
<th>Irr. CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics (mg GAE/g)</td>
<td>119.72±2.37</td>
<td>126.35±2.46</td>
</tr>
<tr>
<td>Total antioxidant activity (mg/mL)</td>
<td>84.23±2.03</td>
<td>89.46±2.11</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE

The data presented in table 2 revealed a significant elevation in glucose concentration associated with a remarkable decline in insulin level in alloxan induced-diabetic rats compared to normal rats. Whereas, diabetic rats fed on raw and $\gamma$-irradiated corn silk had a significant low level of blood glucose and significant high level of insulin compared to alloxan induced-diabetic group.

Table (2): Effect of administration of raw and irradiated CS to alloxan induced-diabetic rats on the level of glucose and insulin of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>D</th>
<th>D+CS</th>
<th>D+ Irr.CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose(mg/dl)</td>
<td>113.04±6.8a</td>
<td>268.58±8.3c</td>
<td>146.20±5.8b</td>
<td>144.12±5.8b</td>
</tr>
<tr>
<td>Insulin(μU/ml)</td>
<td>32.81±3.98a</td>
<td>19.12±4.58c</td>
<td>27.24±3.88b</td>
<td>27.32±4.17b</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n=7).
Values in the same raw with different superscripts are differing significantly at P<0.05.

Table (3) shows a significant increase in the level of MDA associated with a pronounced decrease in GSH content and the activity of SOD and CAT of diabetic rats when compared with the normal group. While diabetic rats received raw or $\gamma$-irradiated CS had a lower concentration of MDA as well as the level of GSH and the activity of SOD and CAT were significantly improved.

Table (3): Effect of administration of raw and irradiated CS to alloxan induced-diabetic rats on the level of MDA, GSH, SOD and CAT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>D</th>
<th>D+CS</th>
<th>D+ Irr.CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (n mol/ml)</td>
<td>191.52±8.48a</td>
<td>397.32±12.81c</td>
<td>251.11±11.12b</td>
<td>249.0±14.11b</td>
</tr>
<tr>
<td>GSH (mg/g tissue)</td>
<td>26.17±1.85a</td>
<td>15.87±1.25c</td>
<td>23.08±1.69b</td>
<td>23.72±1.75b</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>43.94±3.14a</td>
<td>34.22±2.83c</td>
<td>39.52±2.13b</td>
<td>40.31±2.15b</td>
</tr>
<tr>
<td>CAT (U/g protein)</td>
<td>3.62±0.07a</td>
<td>1.66±0.06c</td>
<td>2.91±0.06b</td>
<td>2.98±0.08b</td>
</tr>
</tbody>
</table>

Legend as table 2

In table (4) the serum levels of TC, TG, LDL-C and vLDL-C were elevated with a significant decrease in the serum HDL-C concentration as a result of alloxan administration in comparison with those of the non-diabetic rats. On the other hand, dietary supplementation with either raw or $\gamma$-irradiated CS to alloxan induced-diabetic rats resulted in a reduction in the concentration of lipid contents comparing to diabetic rats.
**Table (4): Effect of administration of raw and irradiated CS to alloxan induced-diabetic rats on lipid contents.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C (mg/dl)</th>
<th>D (mg/dl)</th>
<th>D+CS (mg/dl)</th>
<th>D+ Irr.CS (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>158.95±6.66</td>
<td>225.11±6.75</td>
<td>181.12±5.86</td>
<td>180.39±6.15</td>
</tr>
<tr>
<td>TG</td>
<td>111.18±4.11</td>
<td>177.12±4.41</td>
<td>130.21±4.51</td>
<td>128.17±3.24</td>
</tr>
<tr>
<td>HDL-C</td>
<td>49.78±2.33</td>
<td>32.12±1.98</td>
<td>43.96±2.04</td>
<td>44.52±1.94</td>
</tr>
<tr>
<td>LDL-C</td>
<td>86.94±3.57</td>
<td>157.57±3.97</td>
<td>111.13±3.62</td>
<td>110.24±3.41</td>
</tr>
<tr>
<td>vLDL-C</td>
<td>22.23±1.68</td>
<td>35.42±2.07</td>
<td>26.03±1.51</td>
<td>25.63±1.22</td>
</tr>
</tbody>
</table>

**Legend as table 2**

Rats received alloxan exhibited an elevation in the activity of some liver enzymes (AST, ALT, ALP and GGT) in comparison with those of the control. Conversely, the activity of these liver enzymes was improved in alloxan induced-diabetic rats received raw or γ-irradiated CS compared to diabetic rats (Table 5).

**Table (5): Effect of administration of raw and irradiated CS to alloxan induced-diabetic rats on the activity of some liver enzymes.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C (U/ml)</th>
<th>D (U/ml)</th>
<th>D+CS (U/ml)</th>
<th>D+ Irr.CS (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>23.82±1.21</td>
<td>49.15±2.33</td>
<td>32.15±1.61</td>
<td>32.74±1.54</td>
</tr>
<tr>
<td>ALT</td>
<td>18.84±1.21</td>
<td>38.87±2.42</td>
<td>24.16±1.81</td>
<td>23.91±2.22</td>
</tr>
<tr>
<td>GGT</td>
<td>3.72±0.46</td>
<td>6.21±0.57</td>
<td>4.75±0.61</td>
<td>4.58±0.42</td>
</tr>
<tr>
<td>ALP</td>
<td>9.12±0.91</td>
<td>15.27±1.05</td>
<td>11.26±0.87</td>
<td>11.07±0.94</td>
</tr>
</tbody>
</table>

**Legend as table 2**

**DISCUSSION**

Diabetic patients are prone to the development of atherosclerosis (4). Therefore, the major therapeutic goal of diabetes mellitus is to control blood glucose and lipid levels. Corn silk is a famous traditional herb drug in China and rich in phenolic compounds that can act against hyperglycemic effect (8).

The data presented in Table 1 indicate that the total phenolic contents of raw and γ-irradiated CS was 119.72 and 126.35 (mg GAE/g CS) and the antioxidant activity was 84.23 and 89.46 mg/ml. **Karami et al** (32) reported that the total phenolic content of CS was 118.94 ± 2.78 mg gallic acid equivalent/g of the extract. The results obtained in this investigation revealed that the antioxidant activity may be attributed to the presence of phenolic constituents in CS (33).

**Villavicencio et al** (34) presented that γ-irradiation could result in increasing in the total phenolic contents compared with raw samples and that might be due to the decomposition of some insoluble phenolic compounds.

Alloxan is one of the standard substances used for the induction of diabetes mellitus and it has a destructive effect on the beta cells of the pancreas (35). In the present study, a significant increase in the level of glucose and remarkable decrease in insulin concentration were observed in alloxan-induced diabetic rats. Alloxan, a beta cytotoxin induces diabetes by free radical generation, which causes a massive reduction of the insulin secreting β-cells of the islets of langerhans, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissue (36). Moreover, the adipose tissue and skeletal muscle are unable to uptake glucose from
serum in the absence of insulin so glucose conversion to fat and glycogen is blocked in the adipose tissue and skeletal muscles, resulting in increase of glucose level (37&38).

However, the diabetic group dietary administrated with either raw or γ-irradiated corn silk (CS) showed significant low level of glucose and high insulin concentration in comparison to diabetic rats. Guo et al. (39) reported that the hypoglycemic effect of corn silk could be via increasing insulin level as well as recovering the injured β-cells not by increasing glycogen and inhibiting gluconeogenesis. Also, CS is rich in phenolic compounds, particularly flavonoids that have antioxidant activity and anti-diabetic effect (40).

In the present work, the diabetic rats showed a significant increase in the level of MDA and decrease in GSH content and the activity of SOD and CAT. The increase in MDA and decrease in GSH levels might be due to alloxan induced production of free radicals leading to oxidative stress (41). This result is in agreement with pervious collected data, which referred to accumulation of aldehydic product of lipid peroxidation (MDA) in case of diabetic and general depression of antioxidant status (42&43). Also, the higher levels of free radicals generation resulted in conversion of GSH to its oxidized form (44). SOD and CAT are two major scavenging enzymes that remove the toxic free radical. Reduced activities of SOD and CAT have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals (45).

The results further showed treatment of alloxan-induced diabetic rats with raw and γ-irradiated CS resulted in enhancement of the antioxidant status by increasing the GSH content and the activity of SOD and CAT associated with a significant decrease in the level of MDA. Many studies showed that CS contains phytochemical components which exhibited antioxidant activities and could provide protection against oxidative stress by scavenging free radicals, inhibiting lipid per-oxidation and increasing anti-oxidant enzymes levels (32 & 46).

Alloxan-induced diabetic rats showed a significant high concentration of TC, TG, LDL-C and vLDL-C accompanied by a low level of HDL-C as compared to control. Normally insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic condition lipoprotein lipase is not activated due to insulin deficiency consequential in hypertriglyceridaemia. Also, insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory process due to the uninhibited actions of lipolytic hormones in adipose tissue, which in turns leads to accumulation of lipids such as cholesterol and triglyceride in diabetic rats (38).

On the other hand, treatment of diabetic rats with raw or γ-irradiated CS resulted in remarkable decrease in the level of serum TC, TG, LDL-C and vLDL-C and obvious increase in the concentration of HDL-C. The reduction of lipid contents may be related to the flavonoid compounds of CS that may have potential anti-hyperlipidemic effects. Weggemans and Trautwein (47) reported that flavonoids intake decreased LDL-C and increased HDL-C that may enhance removal of cholesterol from peripheral tissue to liver for catabolism and excretion. Moreover, several studies demonstrated that the ability of isoflavones to decrease serum cholesterol levels may be attributed to an increase in LDL-C receptor activity (48&49).

The results of this study revealed that alloxan administration to rats led to an obvious increase in the activity of serum AST, ALT, GGT and ALP. The increment in the activities of liver enzymes may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (50 &51), which gives an indication on the hepatotoxic effect of alloxan.

However, treatment of alloxan diabetic rats with either raw or γ-irradiated CS enhances the reduction of AST, ALT, GGT and ALP activity. A possible explanation for the effect of CS on the
activities of AST, ALT, GGT and ALP in the serum is that CS may inhibit the liver damage induced by alloxan. Moreover, the reducing effect of CS may be related to their antioxidant effect of the phenolic and flavonoids compounds. Previous study reported that polyphenols can inhibit nitrosation and flavonoids have hepatoprotective activities (52).

CONCLUSION

In general, the present study concluded that corn silk (natural products) may possess anti-diabetic and hypolipidemic effects by decreasing the level of glucose and increasing insulin concentration as well as improving the lipid profile of diabetic rats. Also, this herb could ameliorate some antioxidant enzymes and reduce the activity of some liver enzymes in alloxan-induced diabetic rats. Furthermore, these modulating effects of CS could be attributed to its phenolic and flavonoid compounds.

REFERENCES