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The Effect of Corn Silk Powder on Rats with Diabetic Nephropathy

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Abstract

Corn silk has been used successfully in the traditional management of diseases that are associated with oxidative stress mainly diabetes and diabetic complications such as diabetic nephropathy. The current study aimed to investigate the protective effect of corn silk (CS) powder against diabetic nephropathy. Thirty male Wistar albino rats were randomly divided into five groups (n = 6) as follow; -ve control group fed basal diet, +ve control group fed basal diet and injected with a single dose of Alloxan. Groups 3-5 as the same of group2 and fed on basal diet containing 2.5, 5 and 10% CS powder. Results revealed that CS caused a significant decrease in blood glucose level,urea nitrogen, uric acid, and creatinine. In addition, CS caused a significant increase in insulin level, especially in group 5 which treated with10% compared to other treated group +ve control group. Furthermore, CS caused marked renal and pancreas improvement for damaged that happened due to Alloxan as revealed by alterations in histopathological architectures of kidney and pancreas tissues. In conclusion, CS powder protect rats against Alloxan diabetic nephropathy through its antioxidant, anti-apoptotic, and anti-inflammatory protective mechanisms. So, it may be beneficial to the human beings.

Key Words: Corn Silk, Rats, Diabetic Nephropathy

Introduction

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin (a hormone that regulates blood sugar) or alternatively, when the body cannot effectively use the insulin, it produces. The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes ***(WHO, 2021).***

Glucose homeostasis is extremely altered in patients with diabetic kidney disease (DKD), who are exposed to a high risk of both hyperglycemia and hypoglycemia,bothare associated with increased morbidity and shortened survival. Factors that are associated with an increased risk of hypoglycemia in DKD patients include decreased renal gluconeogenesis, deranged metabolic pathwaysand decreased insulin clearance. On the other hand, decrease glucose filtration and excretion, and inflammation-induce insulin resistance are predisposing factors to hyperglycemic episodes ***(Pecoits-Filho et al., 2016).***

Corn silk (CS) is made from stigmas, the yellowish thread like strands from the female flower of maize. It is a waste material from corn cultivation and available in abundance ***(Maksimović et al., 2005).*** Studies showed that corn silk tea has a function of increasing the urine output, which can help remove the toxins and wastes out, reducing creatinine level. In addition, it also helps remove the excess fluid out, which can help relieve the swelling. High blood pressure, being the most prominent symptom, is reduced with the help of corn silk tea. Although studies suggest that corn silk has a proactive role with nephrotoxicity happened because renal failure ***(Wans et al., 2020).***

Phenolic content in plants is associated with their antioxidant activity, and the flavonoids are the most common phenolics and widely distributed in plants, which are usually effective antioxidant. Based on study that showed the ethyl acetate and n-butanol fractions (ECS and BCS) of corn silk had highest total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, inhibitory activities against α-amylase, α-glucosidase, advanced glycation end-product (AGEs) formation and extracellular matrix (ECM) expression. these results provided scientific insights of the corn silk and confirmed its traditional use of the management of diabetic mellitus (DM) and diabetic nephropathy (DN), and the antioxidant activities could contribute, at least in part, to its traditionally claimed therapeutic benefits ***(Wang and Zhao, 2019).***

A recent study proved that CS is non-toxic in nature. And there have been no histopathological and adverse effects observed at a CS concentration of 8.0% (w/w) consumed for 3 months. This content corresponds to a mean daily CS intake of roughly 9.354 to 10.308 g/day/kg. ***(Wang et al., 2011).***The objective of this study is examining the effects of three levels from corn silk powder on rats with diabetic nephropathy.

Material and methods

Materials:

- Corn silk, collected from local farms in Egypt.
- A total of 30 adult male albino rats (150±10g) Sprague Dawley strain were obtained from of Farm of Experimental Animals, Helwan, Egypt.
- Basal diet (AIN-93), the ingredients purchased from El-gomhouria Pharmacological Company, Cairo, Egypt.

Methods:

Preparation of corn silk powder:

The dried corn silk was collected and separated from the rest of the corn, washed with distilled water and dried at solar energy unit, National Recherche Center, Giza, Egypt. The mild temperature (45-55□C) enabled the dried product to retain its nutrients at described by ***Andritsoset al., (2003).*** Then grinded using a coffee grinded into a fine powder till used for chemical analysis.

Chemical analysis of corn silk:

The polyphenolic content and antioxidants activity of CS was determined according to the method described by ***Brand-Williams et al.,(1995).***

Experimental design:

Rats housed in well aerated cages under hygienic condition and feed on basal diet for one week for adaptation. After this period rats divided into 5 groups (6 rats each). Six rats were served as the -ve control group (G1). Groups from 2-5 injected with single dose of recrystallized Alloxan (120mg\kg) according to ***Ighodaroet al., 2017,*** to induct diabetes. After the appearance of glucose in the urine which was tested using Diabur test diseased rats were classified as follow; G2: +ve control group fed basal diet and groups 3-5 as the same of group 2 and fed basal diet supplemented with CS powder 2.5, 5 and 10%, respectively.

Biological Evaluation:

Feed intake was recorded three times a week, and animals were weighed at the beginning and once a week throughout the experimental period. Body weight gain (BWG), feed efficiency ratio (FER) and organs relative weight were calculated at the end of the experiment according to the method of ***(Chapman et al., 1959).***

Blood collection and biochemical analysis:

At the end of the experimental period (4 weeks), rats were fasted overnight before scarifying and blood samples were collected from each rat and were centrifuged at 3000 rpm for 15 min to obtain the serum for biochemical analysis. Glucose was assayed according to ***Ambadeet al., (1998),*** insulin was assayed according to ***Held, (2019),*** kidney functions such as: urea nitrogen was assayed according to ***Lear, (1950),*** uric acid was assayed according ***Zhao et al., (2008),*** and Creatinine was assayed according to ***Pundir et a l., (2019).***

Histopathological examination of kidney and pancreas:

Specimens from the kidney and pancreas were dissected out, washed with normal saline solution to remove blood and placed in 10% neutral buffered formalin for histopathological examination according to ***Bancroft and Stevens, (1996).*** Histopathological examination was done in Veterinary College, Pathology Department, Cairo University, Egypt.

Statistical analysis:

All data obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data were presented as mean± standard error (SE). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to ***Armitage and Berry, (1987).*** All differences were considered significant if P-values were ($P< 0.05$).

Results and discussion

Polyphenolic content and antioxidants of Corn Silk:

Table 1 revealed that Corn silk had more powerful in phenolic compounds. Results revealed that CS contained high amount of ellagic acid with value 417.10mg\kg, while it has another phenolic component such as: Syringing acid 1.49 mg/kg, Vanillic acid 2.06 mg/kg, p- Coumaric acid 2.25 mg/kg, Quercetin 2.74 mg/kg,

Cinnamic acid 3.63mg/kg, Rutin 4.13 mg/kg, Chlorogenic 4.30 mg/kg, Ferulic acid (5.82mg/kg), Caffeic acid5.02mg\kg, Caffeine 5.15mg/kg, Myricetin acid 16.80 mg/kg, Benzoic acid 23.85mg/kg, Salicylic acid 34.75mg/kg, Naringenin 61.88mg\kg and Kaempferol with value 75.58 mg/kg.

Haslina et al., (2017), said that said that CS contain 8262.93±178.59 µg gallic acid equivalent (GAE)/g. ***Zhou et al., (2019)*** reported that gallic acid had a role a control of the deterioration diabetic nephropathy by decrease the blood glucose, levels of TNF-α and serum creatinine; elevate the activities of antioxidant enzymes; ameliorate the renal pathology; inhibit the up regulation of expression of proteins TLR4, IRAK4, TRAF6, IKK-β, NF-κBp65 and HMGB1 in DN mice.

Result of radical scavenging activity for corn silk which recorded high antioxidants activity 84.07 in 5% result showed in **Table 2.**

Feed Intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER):

Table 3 showed that the FI for each rat was equal in both of positive and negative control groups with mean values 20.2 g/d. Data revealed that, rats fed on diet containing 2.5, 5 & 10% CS decreased the mean values as compared to the controls group, 15.82, 16.32

and 16.64 g/d vs. 20.2 g/d respectively.Regarding BWG, significant increase in BWG% in the positive control group compared to the negative control group with mean values 20.5% and 12%, respectively. Group 3,4 and 5 which treated with 2.5, 5 & 10% CS showed significant increase by mean values 27.16, 30.33 and 35 %, respectively, compared to both ofcontrol groups.Results also revealed that, FER for positive control group was significantly increase compared to negative control group with mean values 1.01 & 0.6 respectively. It was also showed that groups treated 2.5, 5 &10 % CS powder significantly increase compared with positive control group, with mean values 1.72 ,1.9 & 2.1 respectively.

Based on ***Rajagopal and Sasikala (2008),*** reported that significantly higher intake of feed in diabetic group (injected with alloxan) when compared with normal control group, and ***Ha et al., (2018)*** who reported that there is no significantly different among the control group and the corn silk extract treated groups in the mean of feed intake (FI).The result in BWG agreed with ***Muthuraman et al., (2009),*** who approved that the injected with alloxan increased body weight, and disagreewith ***Ojewaleet al., (2020),*** who found the injected rats with alloxan reduced the body weightssignificantly. In corn silk treatment groups ***Ha et al., (2018),***found during the study period, there was no statistically significant difference in body weight among the control and the corn silk extract treatment group.

Relative organs weight of diabetic nephropathy rats treated with CS:

Table 4 recorded there is no significant difference in liver, kidney & heart relative weight between positive and negative control groups for all organs. Results showed also no significant difference among CS treated groups and positive control group. On other hand, spleen relative wight showed nonsignificant different between +ve& -ve control groups, and showed significant decrease in the groups treated with 2.5,5&10% CS with mean values 0.34, 0.36, 0.33%

compared to +ve control group with value 0.27%. Pancreas relative wight showed significant decrease in +ve control group compared to -ve control group with mean values 0.13, 0.19% respectively, while the result in 2.5,5,10% CS doses showed significant increase with mean values 0.19, 0.18 &0.22 % respectively, compared to +ve control group.

Ha et al., (2018) found weights of organs such as heart, liver, spleen, and kidney in the corn silk extract treated groups were compared to those of the control group, no significant difference.

Glucose and insulin levelsof diabetic nephropathy rats treated with CS:

Data in**Table 5** showed that the mean values of serum glucose in positive control group recorded significant increase compared with negative control group, with mean values 432, 82.67 mg/dl, respectively, treated groups with 2.5 & 5 & 10% CS induced significant decrease in serum glucose with values 404.1, 326.1 &289.3 mg/dl respectively.Blood insulin result showed significant decrease in positive control group compared with negative control group with meanvalues 1.39, 9.43 uIU/ml respectively. On other hand treated groups 2.5, 5, 10% CS showed significant increase with mean values 2.51, 3.56, 5.04 uIU/ml respectively, compared with +ve control group. Bestresult observed inthe group treated with 10% CS.

Recent results agreed with ***Ojewaleet al., (2020),*** who found that injected with alloxan rase blood glucose level in rats, alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes by selective necrosis of beta cells in type 1 and type 2 diabetes mellitus, as well as, found significantly decreased in alloxan-induced hyperglycemic mice after administrated with corn silk powder, also increased in insulin secretion, study suggested action of

corn silk on glycemic metabolism is not via increasing glycogen and inhibiting gluconeogenesis but through increasing insulin level as well as recovering the injured β-cells ***(Guo et al., 2009).*** Besides, effect on glucose uptake, CS inhibits α-amylase activity and slow down starch digestion rate and restrained the increase of post-meal blood sugar ***(Chen et al., 2013).***

Kidney Functionsof diabetic nephropathy rats treated with CS:

Results in **Table** 6 showed that nitrogen urea significantly elevated in +ve control group compared with -ve control group with mean values 50 & 5.17 mg/dl respectively, the 2.5% CS level showed nonsignificant decrease, but 5 & 10% CS doses showed significant decrease with mean values 36.5 & 24.16 mg/dl respectively, compared to +ve control group. Uric acid results showed significant increase in +ve control group compared to -ve control group with mean values 227.5 &46.16 mg/dl respectively. 2.5% CS level showed nonsignificant decrease with value 216.5 mg/dl, while 5 & 10 % CS levels showed significant decrease with values 200.3 & 181.2 mmol/L respectively, compared to +ve control group. Creatinine result showed significant increase in +ve control group compared to -ve control group with mean values 3.18, .35 mg/dl respectively, results via treated groups in different levels 2.5, 5 & 10 CS recorded significant increase with mean values of serum creatinine 2.78, 2.43 & 1.75 mg/dl respectively, compared to +ve control group. The best result was 10% level.

The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. A significant increase in total urea and creatinine levels indicated the impaired renal function of alloxan diabetic rats which led to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis. Corn Silk Polysaccharide D3 can lower levels of fasting blood glucose (FBG), kidney index by inhibit the expression level of transforming growth factor beta 1(TGF-β1) of

diabetic rats' kidneys and restrain macrophages in order to protect kidneys of diabetic rats. Corn Silk Polysaccharide D3 can lower levels of fasting blood glucose (FBG), kidney index by inhibit the expression level of transforming growth factor beta 1(TGF-β1) of diabetic rats' kidneys and restrain macrophages in order to protect kidneys of diabetic rats ***(Wen and Yue, 2015; Yuniarti & Lukiswanto, 2016).***

Histopathological Examination:

As shown in **Photos 1-20** Alloxan caused observed damaged in kidney tissue of diabetic animal group revealed shrinkage of capillary tufts with widening of Bowman's space of some glomeruli. The renal tubules showed epithelial cell degeneration, and few intra-luminal albumin casts, with significant necrosis <25% of tubular epithelial lining sc. Vascular damage was appeared as sloughing of endothelial lining and perivascular oedema and mononuclear cells infiltration mainly lymphocytes and macrophages. Also, diabetic rats showed pathological changes of both exocrine and endocrine components. Pancreatic lobules showed atrophy of pancreatic lobules and vacuolation of acinar epithelial ling. The acinar cells were swollen, and small vacuoles were observed in almost all acinar cells. Interlobular ducts were lined with flattened epithelium. Apoptosis of islets of Langerhans's cells which appeared as deeply eosinophilic bodies.

Treatment with Corn Silk Powder attenuated these adverse effects and markedly ameliorated histopathological that caused by Alloxanadministration, especially 10% CS dose. Based on results of the present study, it can be concluded that Corn Silk is a promising diabetic nephropathy protective agent and this protective activity of Corn Silk Powder may be due to its antioxidant, anti-apoptotic, and anti-inflammatory protective mechanisms. So, it may be beneficial to the human beings

Table1: Polyphenolic Compounds Concentration of Corn Silk Powder

Phenolic Compounds	Corn Silk (mg/kg)	Phenolic Compounds	Corn Silk (mg/kg)
Syringing acid	1.49	Caffeine	5.15
Vanillic acid	2.06	Myricetin acid	16.80
p- Coumaric acid	2.25	Benzoic acid	23.85
Quercetin	2.74	Salicylic acid	34.75
Cinnamic acid	3.63	Naringenin	61.88
Rutin	4.13	Kaempferol	75.58
Chlorogenic	4.30	Ellagic	417.10
Ferulic acid	5.82	Caffeine	5.15
Caffeic acid	5.02	Myricetin acid	16.80

Table (2): The Antioxidant Activity of Corn Silk

SN	% DPPH Radical-Scavenging Activity			
	0.5%	1.0%	2.5%	5%
Corn Silk	20.20	41.02	60.33	84.07

Table (3): Effect of Corn Silk Powder on Feed Intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER)

Parameters / Groups	FI (g\d\rat)	BWG%	FER
G1: -ve control	20.20	12.00±01.06d	00.60±00.00d
G2: +ve control	20.20	20.50±03.00c	01.01±00.01c
G3: 2.5%	15.82	27.16±03.73b	01.72±00.01b
G4: 5%	16.32	30.33±01.00b	01.90±00.00b
G5: 10%	16.64	35.00±04.00a	02.10±00.01a

*Mean values are expressed as means ± SE. *Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Table (4): Effect of Corn Silk Powder in Relative Weight

Parameters / Groups	Liver	Kidneys	Heart	Spleen	Pancreas
	%				
G1: -ve control	03.11± 00.09a	00.80± 00.04a	00.28± 00.03a	00.29± 00.02b	00.19± 00.02b
G2: +ve control	03.16± 00.86a	00.95± 00.08a	00.34± 00.03a	00.27± 00.02b	00.13± 00.02c
G3: 2.5%	03.14± 00.18a	00.86± 00.05a	00.34± 00.02a	00.34± 00.02a	00.19± 00.03b
G4: 5%	02.93± 00.10a	00.92± 00.03a	00.31± 00.02a	00.33± 00.03a	00.18± 00.02b
G5: 10%	03.03± 00.39a	00.88± 00.06a	00.33± 00.02a	00.36± 00.05a	00.22± 00.07a

*Mean values are expressed as means ± SE. *Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Table (5): Effect of Corn Silk Powder on Glucose and Insulin level

Parameter / Group	Glucose (mg/dl)	Insulin (uIU/ml)
Group1: -ve control	82.67±01.71e	09.43±00.28a
Group2: +ve control	432.0±01.15a	01.39±00.17d
Group3: 2.5%	404.1±07.14b	02.51±00.22c
Group4: 5%	326.1±01.64c	03.56±00.18c
Group5: 10%	298.3±02.20d	05.04±00.14b

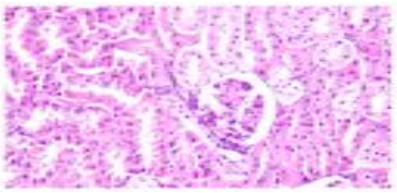
*Mean values are expressed as means ± SE. *Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Table: (6): Effect of Powder Corn Silk on Kidneys Serum

Parameters / Group	Nitrogen urea	Uric acid	Creatinine
	mg/dl		
Group1: -ve control	05.17±01.14d	46.16±01.66d	00.35±00.07d
Group2: +ve control	50.00±03.10a	227.5±03.18a	03.18±00.22a
Group3: 2.5%	47.33±02.60a	216.5±01.17a	02.78±00.21b
Group4: 5%	36.50±02.33b	200.3±04.70b	02.43±00.18b
Group5: 10%	24.16±01.99a	181.2±02.00c	01.75±00.12c

*Mean values are expressed as means ± SE. *Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Histology of Renal Tubules and Glomerulus



Phtoto1:
normal histological structure of renal tubules with intact epithelial lining (G1)

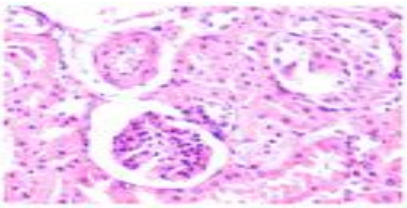


Photo2:
normal histological structure of glomerulus (G1)

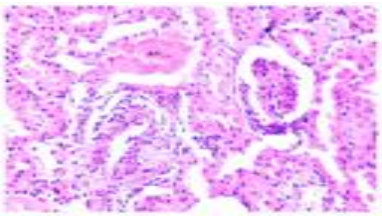


Photo3:
necrosis of tubular epithelial liningwith perivascular oedema and mononuclear cells infiltration (G2)

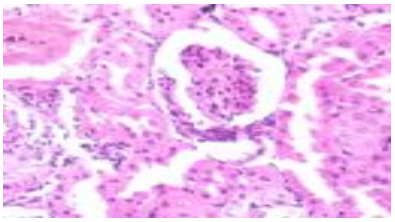
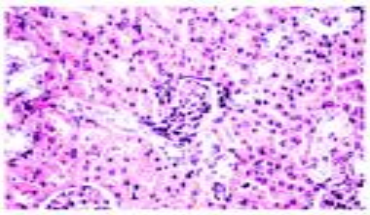


Photo4:
shrinkage of capillary tufts with widening of Bowman (G2)



Phtot5:
swelling of tubular epithelial lining accompanied with narrowing
and occlusion of tubular (G3)

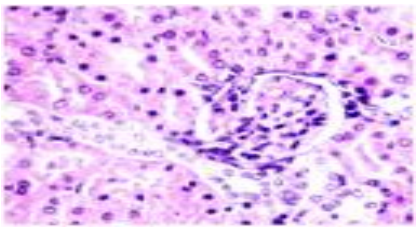


Photo 6:
hypercellularity of capillary tufts and nuclear pyknosis of tubular epithelial lining(G3)

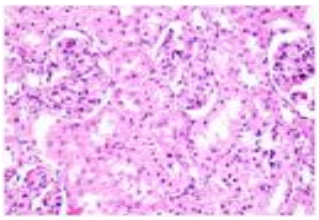


Photo7:
mild swelling of tubular epithelial lining and intra-luminal albuminus eosinophilic droplets (G4)

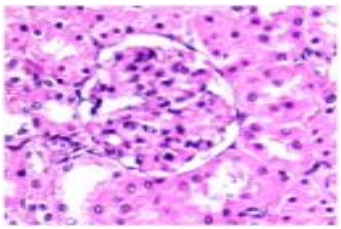


Photo8:
hypercellularity of capillary tufts (G4)

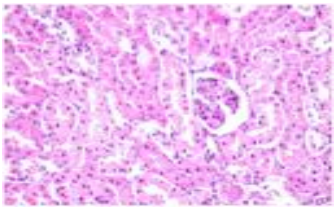


Photo9:
mild swelling of tubular epithelial lining (G5)

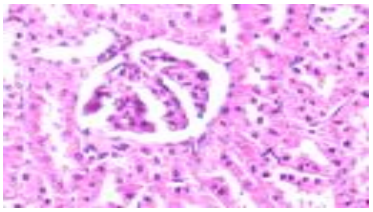


Photo10:
normal circumscribe glomeruli (G5)

Histology of Exocrine Glandular Tissues and Islets of Langerhans

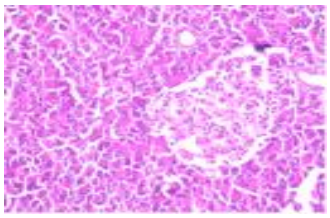


Photo11:
normal histological structure of exocrine glandular tissues (G1)

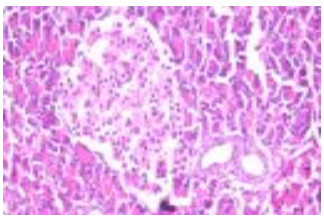


Photo12:
normal histological structure of islets of Langerhans (G1)

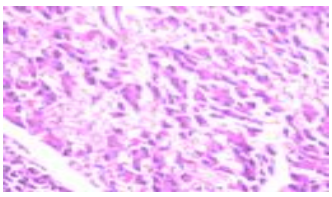


Photo13:
atrophy of pancreatic lobules and vacuolation of acinar epithelial ling (G2)

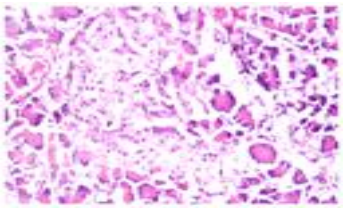


Photo14:
Apoptosis of islets of Langerhans's cells which appeared as deeply eosinophilic bodies (G2)

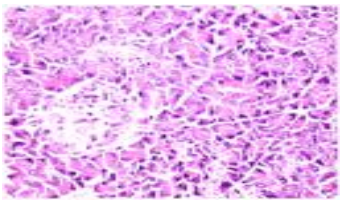


Photo15:
swollen acinar cells with intracellular small vacuoles (G3)

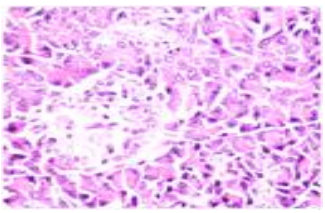


Photo16:
Apoptosis and reduction of islets of Langerhans's cells (G3)

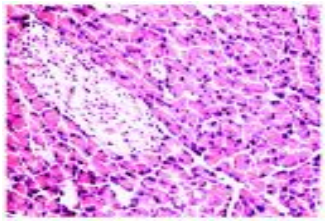


Photo17:
acinar cells appeared with vesicular nuclei (G4)

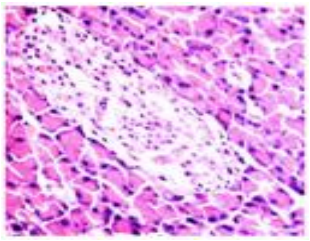


Photo18:
swelling islets of Langerhans's cells(G4)

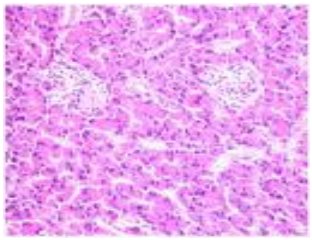


Photo19:
acinar cells appeared with vesicular nuclei (G5)

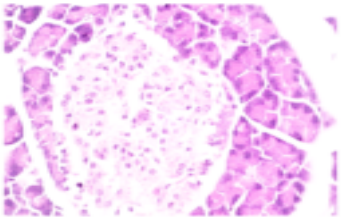


Photo20:
normal islets of Langerhans's cells (G5)

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تأثير مسحوق حرير الذرة على اعتلال الكلى السكري

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تم استخدام حرير الذرة عادة كعلاج شعبي تقليدي للسيطرة على الأمراض وخاصة تلك المرتبطة بزيادة الضغط التأكسدي كمرض السكري وللحد من مضاعفاته كاعتلال الكلى السكري. الهدف من الدراسة الحالية معرفة التأثير الوقائي لمسحوق حرير الذرة ضد اعتلال الكلى السكري. تمت التجربة على ثلاثين فأرًا من فصيلة الألبينو وتم تقسيمهم بشكل عشوائي ل5 مجموعات بمعدل 6 فئران للواحدة, جميع المجموعات تم اطعامهم على النظام الأساسي AIN-93 لمدة 28 يوم, المجموعات من 2-5 تم حقنهم بمادة الألوكسان لاصابتهم بالسكري, وتم تقسيم المجموعات كالأتي: مجموعة 1 : المجموعة المتحكمة السالبة, مجموعة 2: المجموعة المتحكمة الموجبة, مجموعة 3: جرعة 2.5% من مسحوق حرير الذرة, مجموعة 4: جرعة 5% من مسحوق حرير الذرة, مجموعة 5: جرعة 10% من مسحوق حرير الذرة. أظهرت النتائج أن حرير الذرة حقق تقدم ملموس عن طريق التسبب بنقصان معنوي لمستوى سكر الدم, اليوريا, حمض اليوريك, و الكيرياتنين. بالإضافة أن حرير الذرة تسبب بزيادة معنوية لمستوى الأنسولين. وخاصة عند استخدام جرعتي 5% و 10% مقارنة بالمجموعة المتحكمة. كما لوحظ عند اجراء تشريح الأنسجة أن حرير الذرة ساعد على التحسن الإيجابي لكلا من أنسجة الكلى والبنكرياس ضد الضرر الحاصل بسبب الألوكسان.

في الختام: مسحوق حرير الذرة له دور في تحقيق الوقاية ضد اعتلال الكلى السكري التي سببها عقار الألوكسان وذلك يرجع لاحتوائها على مضادات الأكسدة, مضائات السكري, ومضادات الإلتهاب, ولهذا يتوقع أن يكون لها آثر وقائي على الإنسان.

الكلمات المفتاحية : حرير الذرة, فئران, اعتلال الكلى السكري