Hypoglycemic and HypolipidemicEffect of Methanol Extract of Corn Silk (*Zeamays*)in Streptozotocin-induced Diabetic Rats

Ghada [1] M., Eltohami [1] M.S.,Nazik [1] M.M., Rawan [1]B.A.,Rania [1] E.H,Azhari [2*]H.N., Adurahman [3] H.N., and Jessinta S[2].

[1]*Faculty of Pharmacy,Omdurman Islamic University, Sudan*
[2]*Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Malaysia*
[3]*Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Malaysia*

Abstract

Diabetes mellitus was a medical challenge worldwide, and it is a major endocrine disorder responsible for renal failure, blindness, poor metabolic control, and increased risk of cardiovascular disease. The aim of present study was to evaluate the hypoglycemic effect of methanolic extract of corn silk in vivo using albino rats for both type I and type II. Themethanolicextract at doses of 200mg/kg and 400mg/kg possess significant hypoglycemic effect among type-II glucose-loaded diabetic rats ($p<0.001$),whereas the same doses reduced the blood glucosein urea ($p<0.05$), and cholesterol ($p<0.001$) in streptozotocin-induced type-I diabetic rats.The action of corn silk ashypoglycemic agent in type-Imay be due to regeneration of beta-cellsbecause increase of insulin production during the experiment observed. Phytochemical groups were screened by Thin Layer Chromatography (TLC) and positive results were obtained for flavonoids, terpenoids, sesquiterpenes lactones, and saponins. The experimental work showed potential of corn silk as antidiabetic agent. Therefore, determination of bioactive compound(s) warrants further research.

Keywords: Hypoglycemic,hypolipidemic, corn silk, albino rats, phytochemical groups

Introduction

Diabetes mellitus is a group of metabolic disorders affecting a huge number of populations in the world. It is mainly characterized by chronic hyperglycemia, resulting from defects in insulin secretion or insulin action (Patel et al., 2012). Also diabetes mellitus is a major endocrine disorder responsible for renal failure,blindness, poor metabolic control, and increased risk of cardiovascular disease. Many plants are common in the folk medicine for the treatment of diabetes mellitus include*Opuntia* (Cactaceae), *Tecomastans* L. (Bignoniaceae), *Cicerarientinum*(Fabaceae)(Hernandez-Galicia et al., 2002; Howeida et al., 2013). The use of medicinal plants in the treatment of diabetes mellitus is a common practice in wide areas of the Sudan specially the western states, where people depend solely on herb treatment.

Corn plant or corn silk, the genus *Zea* of which *Zea mays*(family: Poaceae) known as Maize silk,is the silky fibers that are found under the leaves and on top of an ear of corn. Corn silks are also known as the stigmas.The stigmas (fine soft, yellowish threads) from the female flowers of maize from 4 to 8 inches long and of a light green, purplish red, yellow or light brown colour, stigmas bifid; the segments very slender, frequently unequal, nearly odorless, faintly sweetish taste. Corn silk has been used in many parts of the world for the treatment of edema, as an oral antidiabetic agent in China for decades as well as for cystitis, gout, kidney stones nephritis, and prostatitis (Wang et al., 2011; Grases et al., 1993).

Traditionally,in Sudanuses many plants including corn silk for controlling of diabetes mellitus. However, in spite of its widespread use, the mechanisms underlying hypoglycemic activity of corn silk

was not yet understood. Therefore, the objective of the present work was to investigate the effects of corn silk on glucose metabolism and attempt to correlate the mechanism of action with phytochemicals present in the plant. Moreover, the effects of corn silk on blood glucose, triglyceride, and cholesterol and insulin secretion, in hyperglycemic rats were also investigated.

Materials & Methods

Plant Material

Corn silk were collected from local markets (Alarabiand Almarkazi) on 13 Sep 2012 and authenticated by the botanists at the National Institute for Medicinal and Aromatic Plant Research, Khartoum Sudan.

Crude methanolic extracts of the corn silk

About 350 g of corn silk were defatted with petroleum ether, followed by chloroform using soxhlet apparatus,and then the residue was extracted with methanol. The solvent was evaporated till dryness using rotatory evaporator.

Phytochemical screening

The plant extracts were subjected to TLC screening for phytochemical groups such as tannins,alkaloids, flavonoids, anthraquinones, glycosides,and saponinsaccording to methods described by Harborne (1998),Parekh et al. (2006), and Wagner and Bladt (2009)with slight modifications. Pre-coated silica gel 60 F_{254}plates were used; these ware glass, aluminum sheets, and plastic sheets (Merck KGaA, Germany). After chromatographic development residual solvent was removed from the plat at r.t. separated spots were visualized using a UV lamp or by spraying with a specific reagents. The methanol extracts solutions were chromatographed on silica plates using solvent system. For sesquiterpenes lactones test used solvent system chloroform: ether (4:1),reagents iodine crystals and vanillin-sulfuric acid, heated for 5 min at 100-110 °C. In flavonoids test, extract was dried and defatted by adding petroleum ether, and then the residue was dissolved in 10 ml ethanol (80%) and filtered. Three ml of the filtrate were taken in test tube to which 4 ml of 1% aluminum chloride ($AlCl_3$) in methanol were added. The second test was done by adding 4 ml of the 1% potassium hydroxide (KOH) to one ml of the filtrate; the observed colours were used for the identification for both tests. Detection of steroids and terpenoids; about 80 ml from prepared extract were evaporated to dryness on a water bath. The residue was extracted with 20 ml chloroform. The chloroform solution was dehydrated over anhydrous sodium sulphate. A 5 ml portion of the chloroform solution was mixed with 0.5 ml of acetic anhydride followed by two drops of con. H_2SO_4. For saponins, about 0.5 g of extract was shaken with water in a test tube.

Experimental animals

For this study, adult male albino rats (70-200 g body weight),were obtained fromSoba Veterinary Research Center, Khartoum, Sudan. The rats used throughout the study were divided into three groups namely; control, standard,and treated groups.Used eight rats in each group and they were fed a diet composed of meat, oil, flour, durra, and water add libitum.

GTT for hyperglycemic rats

This group represents a reversible type II (NIDDM) induced by an intraperitoneal loading dose of 50% glucose at a dose of 2 g/kg. After the eighteen hrs fast, blood samples were obtained from the retro orbital plexus using heparinized capillary tubes (khanna et al., 1992). The samples were collected at 0, 1, 2, and 4 hrs. The 0 time represented the fasting blood samples, while the 2 hrs samples represented the post prandial samples. After collection of blood the calculated loading dose of 2 g/kg of 50% glucose was given intraperitoneally (i/p) to each rat (Konuklugilet al., 1997). Simultaneously a dose of water 10 ml/kg was administered orally to each control rat. The standard was given 10 mg/kgof glibenclamide and the tested group received orally two doses (200,400 mg/kg) of the methanolicextract.The time after administration of doses was noted. Blood samples for the 1, 2 and 4 hrs were collected in the same way.

The glucose concentration was immediately determined in serum by means of plasmatic kit. The same steps were followed for the standard (positive control) and tested groups.

GTT for diabetic rats

This group represented type I (IDDM) induced by an intraperitoneal injection of streptozotocin at a dose of 60mg/kg(Masiello et al., 1998). The same steps were also followed in experiment (2). Samples were collected at 0, 2, 3 and 6 hrs. The concentration of insulin, glucose, cholesterol, urea and triglyceride were determined in serum (Zainab et al., 2009).

Statistical analysis

Data were expressed as means ± standard error of means using paired student's t-test (Mendenhall, 2006)

Results & Discussion

Table 1 shows the effects of methanolic extract of corn silkon the blood glucose in induced hyper glycemic rats. In diabetes type II the dose 200 mg/kg revealed the highest significant glucose lowering effect at 2^{nd}hr ($p<0.001$) as compared to control group.Followed by dose 400 mg/kg which reduced blood glucose significantly ($p<0.05$) throughout the experiment. Whileglibenclamide, showed significant reduction at 2nd h ($p<0.001$) and 4^{th}hr ($p<0.05$).

The effects of a methanolic extract of corn silk: on blood glucose in sterptozotocin induced diabetic rats;on blood urea in sterptozotocin induced hyper glycemic rats;on blood cholesterol in sterptozotocin induced hyper glycemic rats; and on blood insulin in sterptozotocin induced hyper glycemic rats are shown in Tables 2,3,4 and 5 respectively. In streptozotocin diabetic rats (type I) both doses of extract reduced blood glucose in addition to blood urea ($p<0.05$) and significant cholesterol lowering effect ($p<0.001$), but slower in it action as it started at the 3^{rd}hr and continued for the 6^{th} hr. The study showed that dose of 200 mg/kg increase insulin production and the significant increase in insulin observed at dose 400 mg/kg ($p<0.05$) as compared to control groups (Tables 2-5).Increasing insulin level is indicator for possibility of regenerating beta-cells of pancreatic islet, and this effect agree with results obtained by Jain et al. (2010) on *Paspalumscrobiculatum*Linn. The extracts of this produced a dose-dependent fall in fasting blood glucose (FBG). A significant increase in serum insulin level was observed in the treated rats. Serum lipid levels were reversed towards near normal as compared to diabetic control.Chakravarthyand co-workers (1980) mentioned that hypoglycaemic activity of flavonoid fraction from *pterocarpusmarsupium*Roxbdid not show a consistent effect on normal blood sugar levels, but it effectively reversed the alloxan-induced changes in the blood sugar level and the beta-cell population in the pancreas. Aguilar-Santamaría et al. (2009) claimed that the main antidiabetic effect of *Tecomastans* (L.) is due to intestinal alpha-glucosidase inhibition by decreasing the postprandial hyper-glycaemia peak. El-Tantawy and Hassanin (2007) reported that *T. alatus* extract appears to be superior to *T. terrestris* because of restoring the functional β-cells in addition to its hypoglycemic effect and hypolipidemic action in STZ-diabetic animals.

Phytochemical screening for corn silk revealed presence of flavonoids, terpenoids, sesquiterpeneslactones,andsaponins.The formation of a yellow colour with 1% $AlCl_3$ and a dark yellow colour with 1% KOH, indicated the present of flavonoids compounds. In terpenoids test gave dark yellow colour indicated the present of these compounds. Appearance of blue sopts with vanillin-sulfuric acid and brown spots by iodine, indicated the present of sesquiterpenes lactones. Methanolic extract of the study plant was precipitated by using excess amount of ethyl acetate, and formed foam above the liquid surface when shaken the extract with water in a test tube is reliable evidence that saponins are present.The anti-diabetic effect of the studied plants can be attributed to the presence of such phytochemicals and this agreedwithseveral reported,that the biologically active components of plants with hypoglycemic action include flavonoids, polysaccharides, tannins, and terpenoids. Patel et al. (2012) in their study found that plant having antidiabetic activity is mainly due to the presence of the secondary metabolite. In previous study, researchers mentioned that a significant reduction in the level of serum glucose obtained by saponin from *Tribulueterrestris*. Which was the rate of 26.25% and 40.67% in normal mice and diabetic

mice in respectively. The level of serum triglyceride could be reduced 23.35%. The saporin could also decrease the content of serum cholesterol. Serum SOD activity of the mice was increased by the saponin (Li et al., 2002).

Table1. Effect of methanolic extract of corn silkon theblood glucose in induced hyper glycemic rats

Group	Plasma glucose level (mg/dl) Time (hr)			
	0	1	2	4
A (control distilled water 10 ml/kg)	85 ± 5	112±4.4	187± 8.3	154 ± 6.8
B (treated 200 mg/kg)	106± 7	101±8.1	101±8.4**	122 ±15
C (treated 400 mg/kg)	87±5.7	96 ± 5.5	106 ± 8.9*	107 ± 10
D (posative cotrol glibenclamide 10 ml/kg)	96± 4.6	108±4.2	103±6.3**	104±8.6*

(Data are expressed as mean±SEM); * = P<0.05; ** = P<0.001

Table 2. Effect of a methanolic extract of corn silk on blood glucose in sterptozotocin induced diabetic rats

Group	Plasma glucose level (mg/dl) Time (hr)			
	0	2	3	6
A (control distilled water 10 ml/kg)	480 ± 3	496 ± 15	580± 12	651± 30
B (treated 200 mg/kg)	490± 11	407±135	446±19*	383 ± 11*
C (treated 400 mg/kg)	462 ±38	348 ± 33	402±29*	370 ±25*
D (posative cotrol glibenclamide 10 ml/kg)	463±16	460±12	373±30	393±39*

(Data are expressed as mean±SEM); * = P<0.05; ** = P<0.001

Table 3. Effect of methanolic extract of corn silk on blood Urea in sterptozotocin induced hyper glycemic rats

Group	Plasma urea level (mg/dl)			
	0 hr	2 hrs	3 hrs	6 hrs
A (control distilled water 10 ml/kg)	59 ± 9	65 ± 9	83± 11	95± 13
B (treated 200 mg/kg)	50± 6	40 ± 5	51 ± 6*	*55 ± 4
C (treated 400 mg/kg)	48±4	41 ± 4	48± 3*	80 ±14
D (posative cotrol glibenclamide 10 ml/kg)	56±8	37±2*	*54±4	*65±5

(Data are expressed as mean±SEM); * = P<0.05; ** = P<0.001

Table 4. Effect of methanolic extract of corn silk on blood cholesterol in sterptozotocin induced hyper glycemic rats

Group	Plasma cholesterol level (mg/dl) Time (hr)			
	0	2	3	6
A (control distilled water 10 ml/kg)	68± 6	100± 7	733± 63	758± 28
B (treated 200 mg/kg)	112 ±14	85 ± 9	75± 8**	**73±11
C (treated 400 mg/kg)	102± 15	82 ±12	73 ± 10**	63 ±9**
D (posative cotrol glibenclamide 10 ml/kg)	75±3	65±5*	54±4**	50±4**

(Data are expressed as mean±SEM); * = P<0.05; ** = P<0.001

Table 5. Effect ofmethanolic extract of corn silk on blood insulin in sterptozotocin induced hyper glycemic rats

Group	Time (hr)	
	0	2
A (control distilled water 10 ml/kg)	1.18± 18	0.46±10
B (treated 200 mg/kg)	1.35±28	*1.07 ± 8
C (treated 400 mg/kg)	0.29± 7	0.87 ± .22
D (posative cotrol glibenclamide 10 ml/kg)	0.5±29	0.5±29

(Data are expressed as mean±SEM); * = P<0.05; ** = P<0.001

Conclusion

The results obtained in this study strongly suggest that corn silk could be natural source for the treatment of diabetes mellitus. Future work is highly recommended for isolation, identification and elucidation the structures of positive phytochemical groups and correlation of the activity.

Acknowledgement

The authors acknowledge botanists of National Institute for Medicinal and Aromatic Plant Research, Khartoum Sudan for plant identification, and Soba Veterinary Research Center, Khartoum, Sudan for providing experimental animals.

References

Aguilar-Santamaría L., Ramírez G., Nicasio P., Alegría-Reyes C., and Herrera-Arellano A., 2009. Antidiabetic activities of Tecomastans (L.) Juss.exKunth. J Ethnopharmacol., 124(2): 284-288.

Chakravarthy, BK., Gupta S., Gambir SS., and Gode KD. 1980. Pancreatic beta cell regeneration–a novel antidiabetic mechanism of *Pterocarpusmarsupium*roxb. Indian J Pharmacology, 12(2):123-127.

El-Tantawy WH and Hassanin LA, 2007. Hypoglycemic and hypolipidemiceffects of alcohol extract of *Tribulusalatus* in streptozotocin-induced diabetic rats: A comparative study with *T. terrestris* (Caltrop), Indian Journal of Experimental Biology, 45: 785-790.

Grases, F., March, J.G., Ramis, M.,andCosta A., 1993. The influence of *Zeamays* on urinary risk factors for kidney stones in rats, Phototherapy Research, 7(2):146-149.

Harbornej.B., 1998.Phytochemcal Methods: A Guide to Modern Techniqes of plant Analysis, 3rd ed., Chapman and hall, London, UK. Pp. 302.

Hernandez-Galicia E., Aguilar-Contreras A., Aguilar-Santamaria L., Roman-Ramos R., Chavez-Miranda A.A., Garcia- Vega L.M., Flores-Saenz J.L., and Alarcon-Aguilar F.J.2002. Studies on Hypoglycemic Activity of Mexican Medicinal Plants, Proc. West. Pharmacol. Soc., 45:118-124.

Howeida A.M., Idris B.E., Almahdi M.A., Sania A.S., and Abdelwahhab H.M., 2013.Antidiabetic and hypolipidaemic effects of *Cicerarientinum* seeds extracts in hyperglycemic and diabetic rats, Journal of Pharmaceutical and Biomedical Sciences, 30(30): 1046-1052.

Jain S., Bhatia G., Barik R., Kumar P., Jain A., Dixit V.K., 2010. Antidiabetic activity of *Paspalumscrobiculatum*Linn.inalloxan induced diabetic rats, J Ethnopharmacol 127(2): 325-328.

Khanna A.K., Chander R., KapoorN.K.,Singh, C., and Srivastava A.K, 1992. Hypoglyceamic activity of *T.chebulain* in rats, Fitoterapia, 4: 315-356.

Konuklugil B., Deniz G.,Yildiz O., Senoz S., and Saygi S.,1997. Hypoglyceamic effect of *T.Polium* in rats, Fitoterapia. 68(1): 39-41.

Li M., Qu W., Wang H., and Tian C., 2002.Hypoglycemic effect of saponin from tribulusterrestris, Zhong Yao Cai, 25(6): 420-422.

Masiello P., Broca C., Gross R., Roye M., Manteghetti M., Hillaire-Buys D., Novelli M, and Ribes G., 1998. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide, Diabetes, 47(2): 224-229.

Mendenhall W., 2006. Introduction to probability and statistics/ W. Mendenhall, R. J.Beaver, B. M. Beaver.-12th ed.-Belmont, CA: Thomson/Brooks/Cole, 743.

Parekh J., Karathia N., and Chanda S. 2006.Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata*L. Bark, African Journal of Biomedical Research, 9, 53 – 56.

PatelD, Kumar R, Laloo D, Hemalatha S.2012. Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. A sian Pac J Trop Biomed, 2(5):411-420

Wagner H. and Bladt S., 2009. Plant Drug Analysis: A Thin Layer Chromatography Atlas, 2ed ed., Heidelberg, Germany, Pp. 384.

Wang C., Zhang T., Liu J., Lu S., Zhang C., Wang E., Wang Z., Zhang Y., and Liu J., 2011. Subchronic toxicity study of Corn silk with rats, Journal of ethnopharmacology, 137(1): 36-43.

Zainab K., Rehman SZ,,Tajuddin, AbdulLatif, and Jahan N., 2009. Hypoglycemic activity of *Berg-e-Arusa*and*Shoneez*, Indian Journal of Traditional Knowledge, 8(3):410-412.