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Hypoglycemic Effect due to Insulin Stimulation with *Plantago major* in Wistar Rats

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Abstract

Background: Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia, which causes secondary pathophysiological changes in multiple organ systems. Several times the Plantaginaceae family have been reported as crude drugs with hypoglycemic effect mainly attributed to their high fiber content.

Objective: The current study was hypothesized to investigate the beneficial effects of methanol extract from the aerial part of *Plantago major* (Pm) on hyperglycemia-mediated oxidative stress and inflammatory responses Alloxan induced diabetes mellitus, and in normal rats.

Materials and methods: Experimental diabetes was induced by intraperitoneal injection of 100 mg/kg body weight Alloxan. Normal and Diabetic rats were administered with distilled water, methanol extract of *Plantago major* and glibenclamide, and then, compared in Oral Glucose Tolerance Test (OGTT).

Results: Methanol extract of *P. major* was evaluated in OGTT in either normal or moderate or severe Alloxan induced diabetic rats (100 mg/kg –by intraperitoneal injection-). Oral administration of methanol extracts at doses of 500mg/kg body weight showed that the extract promotes glucose uptake in rats with efficient insulin secreting pancreas (that have pancreatic cells capable of secreting insulin).

Conclusion: The present study demonstrates that *Plantago major* exerts antidiabetic activity by stimulating secretion of insulin and producing a hypoglycemic effect. These results suggest that the methanol extract from the entire Pm plant will be useful in the treatment of patients with impaired glucose tolerance.

Keywords: *Plantago major*; Hypoglycemia effect; Glucose tolerance; Insulin secretion

Abbreviation:

DM: Diabetes mellitus; Pm: *Plantago major*; OGTT: Oral Glucose Tolerance Test; SCFAs: Short Chain Fatty Acids.

Introduction

Diabetes mellitus (DM) comprises a group of frequent metabolic disorders that share the common phenotype of hyperglycemia. Depending on the cause of DM, the factors that contribute to hyperglycemia may be insulin secretion deficiency, insulin resistance, or both, which lead to carbohydrate, lipid, and protein metabolic anomalies [1].

Currently used controlling agents in the treatment of DM are sulfonylureas, biguanides, and thiazolidinedione derivatives. *Plantago major* (Pm) is a perennial plant belonging to the family Plantaginacea [2], extensively used in Latin American countries for the treatment of varied conditions, from peptic ulcer disease to diabetes mellitus. Its hypoglycemic effect has mainly been attributed to its high fiber content [3].

The American Diabetes Association (ADA) recommends an intake 20-35 gr/day of both soluble and insoluble fiber to better control glycemic and insulin levels. It seems that the soluble fraction is the most effective in the control of glycaemia. A study in the New England Journal of Medicine showed that people with diabetes who ate 50 grams of fiber a day (particularly soluble fiber) could control their blood glucose better than those who ate far less. However, the mechanisms between high fiber intake and improved glycemic management remain undefined [4].

The benefits of fiber on glucose control might be linked to delayed gastric emptying; decreased absorption of glucose trapped by the fiber's viscosity, and thus, less accessible to the action of pancreatic amylase; and short-chain fatty acids (SCFAs) production (formed by the fermentation of the fiber by gut microbiota) [5]. In particular, propionic and butyric acids (SCFAs) were shown to decrease plasma glucose levels, insulin resistance as well as inflammation, and an increase in protective Glucagon-like peptide-1 (GLP-1) secretion, stimulating secretion of insulin [6]. *In vitro* studies have shown that fermentation by colonic bacterial flora of *Plantago ovata* seeds produce an increase of SCFA, particularly butyrate. This type of fiber is fermented slowly along the entire colon, thus capable of maintaining high levels of butyrate in the distal colon. It has been shown that butyrate stimulates insulin secretion and reduces TNFa levels [5]. A

study in 2013 revealed that the Pm seed mucilage had greater release of propanol, an insulin secretion stimulant, compared to standardized tablets such as Tragacanth and HPMC-K4M (Hydroxypropyl Methyl Cellulose K4M) [7].

The effect of extracts of Pm on glucose tolerance is currently unknown beyond anecdotal data. In this study, we evaluated the effect of methanol extract from the aerial part of *Plantago major* (Pm) in control and diabetic Wistar rats were evaluated in Oral Glucose Tolerance Tests (OGTT).

Materials and Methodology

Extraction of *Plantago major*

The aerial parts of Pm were used, as described in Abud and collaborators (Abud et al.) which were collected from Greater Mendoza, Argentina. The identification of the plant was conducted at the National University of San Luis by Dr. Luis Del Vito. Voucher No. 490.

Preparation of methanol extract

The plant material was dried at room temperature sheltered from light, to be milled to fine powder and then processed. The vegetable material (260 g.) Was macerated with n-hexane three times for 72 h. The extracts were collected and concentrated to dryness using a rotary vacuum evaporator to give the n-hexane extract (6 g). The plant material after being macerated with n-hexane, methanol was added, left at room temperature for 72 h, to carry out the corresponding solid-liquid extraction, filtered and the operation repeated on three occasions. Filtrate liquids and concentrated to dryness using distillation under reduced pressure to give the methanolic extract (21 g).

HLPC analysis

The methanolic extract (21 g) was chromatographed on silica gel 60 H, using as chloroform: methanol solvent in the following ratio 99:1; 98:2; 96:4; 92:8; 88:12; 85:15; 80:20; And 75:25, consecutively. All the fractions were tested by thin layer chromatography (TLC) using benzene: dioxane: acetic acid (90:25:4) and chloroform: methanol (7: 3) as the developing solvent. The developer used was anisaldehyde, in order to, visualize iridoid components characteristic of the genus and species.

The fractions eluted with chloroform: methanol (98:2) and (96:4) were chromatographed on 60 G silica gel, using chloroform as solvent in increasing concentrations of methanol. In both, the presence in two-component thin-plate chromatography is observed. The 1 H-NMR spectrum of the mixture indicates the presence of hydroxylated triterpenes.

Fractions eluted with chloroform: methanol (88:12) and (85:15), reveal the presence of a white solid (9 mg). The only study performed was H1-NMR in deuterated water. Signals typical of carbohydrates, which respond to structures of di-trisaccharides (probably in the form of O-alpha-D-galactopyranosyl (1-6) -beta-D-fructofuranosyl-alpha-D- Glucopyranoside typical of *Plantago major*.)

Fractions eluted with chloroform: methanol (92: 8), (88:12) and (85:15) revealed by thin layer chromatography using as an anisaldehyde developer the presence of an iridoid. Previous studies by R. Taskova (Taskova et al.), indicate the aucubine-type iridoid as a component of *P. major*.

Experimental animals

We worked with 30 male Wistar rats, weighing 300-450 g ranging between 4 and 6 months of age, water ad libitum, exposure to light of 12 hours and kept in special cages in the animal care center of the School of Medicine of the UNCuyo. Animal care and experimental protocols were approved by the National University of Cuyo School of Medicine's Committee of Ethical Conduct in the Care and Use of Experimental Animals (Comité Institucional para el Cuidado y Uso de Animales de Laboratorio -CICUAL-).

Development of diabetes in rats

Two groups had experimentally induced diabetes by injecting Alloxan intraperitoneally at doses of 150 mg/kg body weight for the groups comprised of 4 experimental diabetic control rats and 4 experimental moderate diabetic rats having fasted 8 hours; a single high dose of 200 mg/kg for the experimental severe diabetic group, which were 4 rats, also having fasted 8 hours. Seven days after the administration of Alloxan, the blood glucose meter test was realized to determine oral glucose tolerance, obtaining blood samples from each animal by puncture of the tail vein.

Alloxan (urea derivative) is a very unstable hydrophilic chemical compound which has a molecular shape like glucose. Due to their hydrophilicity, both glucose and Alloxan do not penetrate the lipid bilayer of the plasma membrane. However, the shape of the alloxan molecule is so similar to that of glucose that the glucose transporter, GLUT2, which is the pancreatic beta cell transmembrane transporter accepts this molecule as a glucose analog and transports it into the beta cell pancreas´s cytosol [8]. Selective beta cell necrosis is due to the production of free radicals in the cytosol by the Alloxan [9-11]. Therefore, we decide to use Alloxan because it is the most commonly used chemical for diabetes mellitus induction and is a well-known diabetogenic agent widely used to induce Moderate diabetic animals [12].

Biochemical parameters

Glycaemia was determined by use of colorimetric method (glucose oxidase) during the Oral Glucose Tolerance Test (OGTT). Before OGTT, rats were made to fast 15 hours, distilled water was administered to the Control and Dbcontrol groups, and glybenclamide methanol extract was given orally 30 minutes before administration of glucose for the corresponding group. Glucose (1.25 g/kg) was administered orally to each rat. Blood samples were taken from tail veins every 30 minutes: starting 30 minutes right before the administration of the extract, which will be recorded as -30 min within the test, 0 minutes (just before the administration of glucose), 30, 60, 90 and 120 min for assaying glucose.

OGTT curves reflect the efficiency of the body to eliminate excess glucose after an oral glycemic load, therefore, it is a dynamic test. OGTT curves mimic glucose homeostasis and insulin response as in physiological conditions. However, it is important to recognize that glucose tolerance and insulin sensitivity are not equivalent concepts.

Experimental design

The rats were divided into 6 groups, 4 rats in each:

Group 1: Normal control rats received 0.9% saline (Con)

Group 2: Normal rats treated with 500 mg/kg of Pm (N+Pm).

Group 3: Normal rats treated with glibenclamide 5 mg/kg, (used as standard reference drug) (N+Gb).

Group 4: Experimental Diabetic Control rats received 0.9% Saline (DbCon).

Group 5: Experimental Severe Diabetic rats treated with 500 mg/kg of Pm (SevDb+Pm).

Group 6: Experimental Moderate Diabetic Control treated with 500 mg/kg of Pm (ModDb+Pm).

Results

The results of blood glucose measurements obtained from the OGTT and the respective curves of different Normal groups (Con, N +Pm and N+Gb) seen in Graph 1 and Table 1.

In Graph 1 and Table 1, you can see the results of oral glucose tolerance group between Con, N+Pm and N+Gb.

Normal control rats received 0.9% saline (Con)						
Rat	**-30**	**0**	**30**	**60**	**90**	**120**
1	98	121	156	139	120	129
2	85	127	148	119	131	122
3	109	114	154	158	137	128
4	94	97	119	150	141	121
CI	96.5 +/-9.75	114.75 +/-12.71	144.25 +/- 16. 83	141.5 +/-16.56	132.25 +/-8.96	125 +/-4
Percentage decrease of glycemia				1.91 +/-11.48%	8.32 +/-6.12%	13.35+/-2.77%
Normal rats treated with 500 mg/kg of Pm (N+Pm).						
Rat	**-30**	**0**	**30**	**60**	**90**	**120**
1	90	95	121	102	103	95
2	98	93	154	148	119	110
3	86	104	138	142	127	109
4	89	98	128	131	119	102
CI	90.75 +/-5.02	97.5 +/-4.7	35.25 +/- 14.03	130.75 +/-20.01	117 +/- 9.86	104 +/- 6.87
Percentage decrease of glycemia				3.33+/-14.79%	13.5+/-7.29%	23.11+/-5.05%
Normal rats treated with glibenclamide 5 mg/kg. orally (used as standard reference drug). (N+Gb)						
Rat	**-30**	**0**	**30**	**60**	**90**	**120**
1	91	95	121	109	101	95
2	94	117	160	125	130	118
3	98	100	165	144	142	116
4	95	102	135	130	121	100
CI	94.5 +/-2.83	103.5 +/-9.28	146 +/-19.97	127 +/-14.16	123.5 +/-16.95	107.25 +/-11.24
Percentage decrease of glycemia				13.01 +/-7.69%	15.41 +/- 11.6%	26. 54 +/- 7.69%

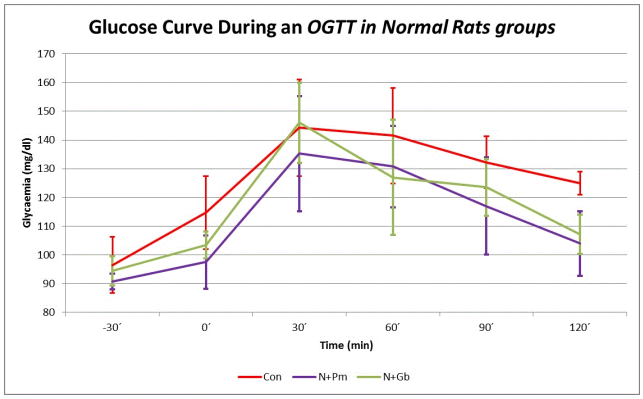
Table 1: OGTT in Normal Rats groups.CI: confidence Interval.

Table 2 shows the results between the Experimental Diabetic Groups of their OGTT. Graph 2 shows the shapes of the curves of OGTT among SevDb+Pm, ModDb+Pm and DbCon and Con.

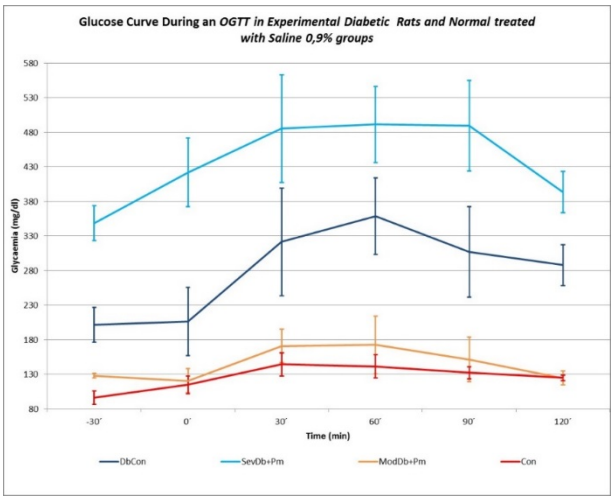
Graph 3 shows the results between Experimental Diabetic Rats (ModDb+Pm and DbCon) and Normal (Con and N+Pm) treated with Saline 0.9% groups.

Experimental Diabetic Control rats received 0.9% Saline (DbCon)						
Rat	**-30**	**0**	**30**	**60**	**90**	**120**
1	217	215	313	361	311	278
2	206	195	328	357	309	296
3	196	204	330	363	305	298
4	187	211	315	353	303	298
CI	201.5 +/-12.66	206.25 +/-8.59	321.5+/-8.56	358.5 +/-4.36	307 +/ 3.57	288 +/-0.19
Percentage decrease of glycemia					14.37% +/-0.1437%	17.92 +/-1.52%
Experimental Severe Diabetic rats treated with 500 mg/kg of Pm. (SevDb+Pm)						
Rat	-30	0	30	60	90	120
1	322	399	460	560	537	3<:9
2	356	383	416	422	434	412
3	381	496	600	493	557	413
4	335	410	465	490	430	400
CI	348.5 +/-25.28	422 +/- 49.45	485.25 +/-78.01	491.25+/-55.22	489.5+/-65.57	393.5+/- 29.64
Percentage decrease of glycemia					**0.36 +/-13.34%**	**15.82 +/-2.37%**
Experimental moderate diabetic control treated with 500 mg/kg of Pm. (ModDb+Pm)						
Rat	-30	0	30	60	90	120
1	123	97	170	123	115	119
2	130	139	196	211	190	140
3	128	118	138	151	136	120
4	131	128	180	205	165	120
CI	128 +/-3.48	120.5+/-17.5	171 +/-23.98	172.5 +/-41.77	151.5 +/-32.19	124.75 +/-9.97
Percentage decrease of glycemia					12.7 +/-18.66%	27.69 +/-5.78%

Table 2: Results between the Experimental Diabetic Groups of their OGTT.

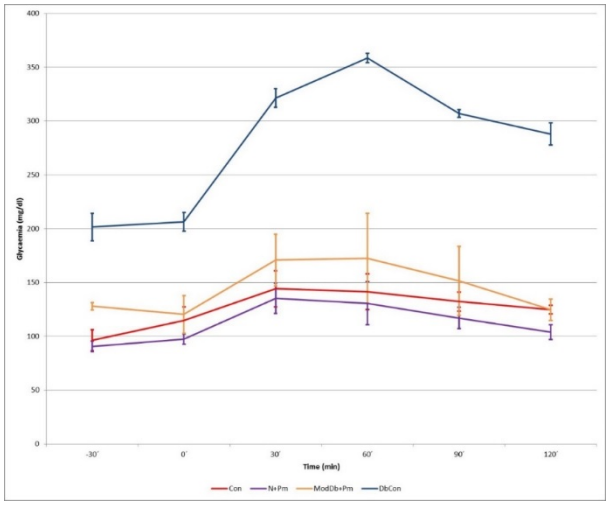


Graph 1: Glucose Curve during an OGTT in Normal Rats groups. Con, Normal control rats received 0.9% saline; N+Pm, Normal rats treated with 500 mg/kg of Pm; N+Gb, Normal rats treated with Glibenclamide 5 mg/kg.



Graph 2: Glucose Curve During an OGTT in Experimental Diabetic Rats and Normal treated with Saline 0.9% groups. DbCon, Experimental Diabetic Control rats received 0.9% Saline; SevDb +Pm, Experimental Severe Diabetic rats treated with 500 mg/kg of Pm; ModDb+Pm, Experimental Moderate Diabetic treated with 500 mg/kg of Pm; Con, Normal control rats received 0.9% saline.

Graph 3, which shows the shapes of the curves of OGTT among Con, N+Pm and ModDb+ Pm, highlights that there is a statistical significant difference between ModDb+Pm and N+Pm at 120 min, and there is no statistical difference between ModDb+Pm and Con. That gives us the idea that N+Pm has a relative hypoglycaemia compared to ModDb+Pm and Con due to a normal pancreas plus the stimulation of insulin secretion by the PM. Furthermore, there is no Statistical difference between these groups in their percentages of decrease glycaemia.



Graph 3: Glucose Curve During an OGTT in Experimental Diabetic Rats and Normal treated with Saline 0.9% groups. Con, Normal control rats received 0.9% saline; N+Pm, Normal rats treated with 500 mg/kg of Pm; ModDb+Pm, Experimental Moderate Diabetic rats treated with 500 mg/kg of Pm.

Discussion

Type 2 DM is a metabolic syndrome characterized by decreased insulin secretion and insulin resistance. Alloxan induced type 2 diabetic rat models were used in the study as it resembles decreased insulin secretion in DM.

The Alloxan exerts its diabetogenic action when administered parenterally: intravenously, intraperitoneally or subcutaneously. The required dose of Alloxan for induction of diabetes depends on the animal, route of administration and nutritional status species. The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg body weight. When Alloxan is administered intraperitoneally or subcutaneously, the effective dose is 2-3 times higher. An intraperitoneal dose below 150 mg/kg body weight may be insufficient to induce diabetes in the rat. Fasted animals are more susceptible to Alloxan, while increasing blood glucose provides partial protection [8]. The preferred route of administration is intraperitoneal [13].

Moderate diabetic animals are recommended for use in testing drugs that want to know if they have a stimulating effect of insulin secretion. The optimal dose of Alloxan for severe diabetic rats via intraperitoneal injection appears to be a single high dose of 200 mg/kg. A high percentage of these animals (approx. 70%) develop insulin-dependent diabetes mellitus [13]. In this project, we wanted to compare groups of rats with moderate experimental diabetes or non-insulin dependent diabetes mellitus (index of 130 - 250mg/dl of glucose) and groups of rats with severe diabetes or insulin-dependent diabetes mellitus (index> 250 mg/dl of glucose) to compare the stimulation effect of Pm in the insulin secretion in different groups [11-15].

If we see Graph1 we can note that at time 120' there is a statistically significant difference in the treated groups N+Pm and N+Gb in comparison to Con, resulting in a $p < 0.05$. Also, note in Table.1, N+Pm

had a decrease of glycemia of 23.11 +/- 5.05% from the maximal peak of glycaemia at 60min, the N+Gb of 26.54 +/- 7.69% and the Con decreased 13.35 +/- 2.77%. Such decreases in blood glucose of their respective peaks are also statistically significant between N+Pm and N +Gb in comparison to Con. These shows that the Pm has a very similar effect in the OGTT like Glibenclamide.

In Table 2, we note that the percentage of decreased glucose of MdDb+Pm is 27.69 +/- 5.78% at 120 min of the peak at 60 min compared to the other Diabetic groups, SevDb+Pm and DbCon with decreases of 15.82 +/- 2.37 % and 17.92 +/- 1.51% at 120 min respectively of their maximum peak blood glucose levels at 60 min. Such decreases in blood glucose of their respective peaks are also statistically significant between ModDb+Pm and the other diabetics groups. This difference between the proportions is statistically significant and gives the pattern of more Insulin stimulation in ModDb +Pm.

In Graph 2, we can see that at the beginning of OGTT that only at -30 min there is an important difference between Con and Md+Pm, but then after the administration of Pm there is no important significant difference at any time between those groups.

Conclusion

Our study clearly indicated that Pm has a potent hypoglycemic effect in moderate Alloxan induced diabetic rats and normal rats. The hypoglycemic effect is produced by the stimulation of insulin secretion. The beneficial effect of Pm was comparable with the antidiabetic drug Glibenclamide. Fibers present in Pm may act as potential candidates in slowing the progression of diabetic complications. Thus, the dietary supplementation of Pm may be helpful for the management of diabetes complications and it could be developed as a new food additive or drug ingredient for the prevention of diabetes mellitus.

Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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