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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 [5]. 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 100mg/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 the efficient insulin-secreting pancreas 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.

GRAPHICAL ABSTRACT

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Short-chain fatty acids (SCFAs), Glucagon-like peptide-1 (GLP-1). The fiber from the *Plantago major* fermented slowly along the entire colon, thus capable of maintaining high levels of butyrate and propionic acid in the distal colon. The butyrate and propionic stimulates insulin secretion and stimulate Glucagon-like peptide-1 (GLP-1) secretion. Those actions end in the decrease of glucose levels.

Keywords

Plantago major; Hypoglycemia effect; Glucose tolerance; Insulin secretion

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 Plantaginaceae [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 TNF α 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).

Biochemical parameters

Glycemia 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)
- Group2: 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 No. 1 and table No1.

Graphic No3 shows the results between Experimental Diabetic Rats (*ModDb+Pm* and DbCon) and Normal (Con and N+Pm) treated with Saline 0,9% groups.

Graph No. 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.

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. He 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. He 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]. He 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-independent diabetes mellitus [13]. In this project, we wanted to compare groups of rats with moderate experimental diabetes or noninsulin 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 significance different in the treated groups N+Pm and N+Gb in comparison to Con, with a p value <0.05. Also note in Table N1 the group N+Pm had a decrease of glycemia of 23.11 $\pm$ 5.05% from the maximal peak of glycaemia at 60min, the N+Gb of 26.54 $\pm$ 7.69% and the Con group decreased 13.35 $\pm$ 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. Hese 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 $\pm$ 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 $\pm$ 2.37 % and 17.92 $\pm$ 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 groups, but then after the administration of Pm there is no important significant diffearence 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.

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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.

Abbreviations:

DM	Diabetes mellitus
Pm	Plantago major
OGTT	Oral Glucose Tolerance Test
SCFAs	short-chain fatty acids

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Graph No.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 No2.

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.

GraphNo3.

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

Table No. 1.

OGTT in Normal Rats groups.CI: confidence Interval.

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 +/- 16,82	141,25 +/- 16,56	132,25 +/- 8,95	125 +/- 4
Percent decrease of glycemia				1,91 +/- 11,48 %	8,32 +/- 6,12 %	13,35 +/- 2,77 %
Normal rats treated with 500mg/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	135,25 +/- 14,02	130,75 +/- 20,01	117 +/- 9,86	104 +/- 6,84
Percent decrease of glycemia				3,33 +/- 14,79%	13,5 +/- 7,29 %	23,11 +/- 5,05 %
Normal rats treated with glibenclamide 5 mg/kg orally (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	138	130	121	100
CI	94,5 +/- 2,83	103,5 +/- 9,28	146 +/- 19,97	127 +/- 14,15	123,5 +/- 16,94	107,25 +/- 11,24
Percent decrease of glycemia				13,01 +/- 7,69 %	15,41 +/- 11,6 %	26,54 +/- 7,69%

Table No. 2.

OGTT in Experimental Diabetic Rats groups. CI: Confidence Interval.

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	280
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
Percent decrease of glycemia.					14,37% +/- 0,1437%	17,92 +/- 1,51%
Experimental Severe Diabetic rats treated with 500 mg / kg of Pm. (SevDb+Pm)						
Rata	-30'	0'	30'	60'	90'	120'
1	322	399	460	560	537	349
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
Percent decrease of glycemia.					0,36 +/- 13,34%	15,82 +/- 2,37%
Experimental Moderate Diabetic Control treated with 500 mg / kg of Pm. (ModDb+Pm)						
Rata	-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
Percent decrease of glycemia.					12,7 +/- 18,66%	27,69 +/- 5,78%