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Original

Hypoglycemic effect of cooked *lupinus mutabilis* and its purified alkaloids in subjects with type-2 diabetes

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Abstract

Developing countries are experiencing an epidemic of chronic non-communicable chronic diseases with high socio-economic costs. Studies of traditional foods with beneficial health properties could contribute to diminish these problems. Legumes rich in proteins like *Lupinus mutabilis* decreases blood glucose and improves insulin sensitivity in animals and humans. We report the results of a phase II clinical trial conducted to assess the role of cooked *L. mutabilis* and its purified alkaloids on blood glucose and insulin in volunteers with diabetes. Results indicate that consumption of cooked *L. mutabilis* or its purified alkaloids decreased blood glucose and insulin levels. The decreases in serum glucose concentrations from base line to 90 minutes were statistically significant within both treatment groups; however, there were not differences between groups. Serum insulin levels were also decreased in both groups however the differences were not statistically significant. None of the volunteers in either group presented side effects.

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Key words: Lupinus mutabilis. *Hypoglycemia. Diabetes. Ecuador. Alkaloids.*

EFECTO HIPOGLICEMIANTE DE *LUPINUS MUTABILIS* COCINADO Y SUS ALCALOIDES EN SUJETOS CON DIABETES TIPO-2

Resumen

Los países en vías de desarrollo están sufriendo una epidemia de enfermedades crónicas no transmisibles con costos socio-económicos grandes. El estudio de alimentos tradicionales con efectos benéficos para la salud podría contribuir a solucionar estos problemas. El consumo de leguminosas ricas en proteínas como *Lupinus mutabilis* disminuye las concentraciones de glucosa e insulina en animales y humanos. Aquí se reportan los resultados de un estudio clínico fase II realizado para evaluar la eficacia de *L. mutabilis* cocido y de un extracto de alcaloides de *L. mutabilis* en las concentraciones de glucosa e insulina sanguíneas en voluntarios con diabetes. Los resultados indican que el consumo de *L. mutabilis* cocido o de sus alcaloides purificados disminuyeron las concentraciones de glucosa e insulina en sangre. La disminución en las concentraciones de glucosa sanguínea entre la línea basal y los 90 minutos después del tratamiento fueron estadísticamente significativas dentro de cada grupo de tratamiento, sin embargo no hubieron diferencias entre los grupos. Los niveles de insulina también disminuyeron en ambos grupos pero las diferencias no fueron estadísticamente significativas. Ninguno de los voluntarios presentó efectos adversos a los tratamientos.

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Palabras clave: Lupinus mutabilis. *Hipoglicemia. Diabetes. Ecuador. Alcaloides.*

Introduction

Chronic non-communicable diseases such as diabetes are increasing public health problems worldwide.[1] The prevalence of these diseases will increase drastically in the coming years particularly in developing countries. Diabetes is expected to affect approximately 300 million people by 2025.[2] In Ecuador, diabetes has been among the main causes of death in the last decade.[3] The social and economic cost to treat diabetes is very high especially in developing countries where limited resources are allocated by governments for health care and most people are not able to afford current treatments.[4] It is important then to establish preventive strategies, including education, directed at improving life style behaviours such as increased physical activity and consumption of adequate, healthy diets.[1] In this regard,

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data indicate that consumption of protein could improve insulin and glucose homeostasis.[5] Recently, several studies, including our own, have shown that consumption of protein rich legumes decreased blood glucose and improved insulin sensitivity in animal models of diabetes and humans with glucose abnormalities.[6] Thus, consumption of raw *Lupinus mutabilis* by individuals with glucose abnormalities significantly decreased their blood glucose levels. Raw *Lupinus* effects were greater in those subjects with higher basal glucose levels. A statistically significant reduction in insulin levels was also observed after raw *Lupinus* intake. Furthermore, treatment with raw *Lupinus* improved insulin resistance in subjects with glucose abnormalities.[6] In that study approximately 30% of the participants presented minor side effects. Research on health related properties of foods could be an important way to prevent and treat chronic non-communicable diseases.

Considering the hypoglycemic effects of raw *L. mutabilis* as well as its potential toxicity, the objective of the present study was to evaluate separately the effect of oral administration of cooked *L. mutabilis* without alkaloids and an extract of alkaloids from *Lupinus* on plasma glucose and insulin concentrations.

Subjects and methods

The Human Subjects Protection Committee at the Universidad San Francisco de Quito approved this study. Each participant signed an Informed Consent form after receiving an explanation of the study and its possible consequences.

Subjects and study design

A phase II clinical trial was conducted with volunteers recently diagnosed with type-2 diabetes according with American Diabetes Association criteria and with a glucose level at an initial screening greater than 110 mg/dL with a rapid strip test (Accu check advantage), Roche Quito-Ecuador). Participants were recruited from the Endocrinology Unit in a municipal health care center in Quito-Ecuador. Eligible subjects were randomly assigned to consume cooked *L. mutabilis* or an extract of alkaloids from *L. mutabilis* in a proportion of two (*Lupinus*) to one (alkaloids).

Preparation of cooked Lupinus mutabilis

Lupinus mutabilis was obtained as previously described.[7] Raw dried beans were soaked overnight with tap water, washed 3 times and cooked in a ratio of 3:1 water to beans for one hour. After cooking, the beans were subjected to debittering by agitation at 30°C for 72 hours.[8] At the end of the debittering process, the beans were dried on a cement platform until the beans contained 13% water. Subsequently the beans with skin on were milled to a 300-micron powder and encapsulated in gelatin capsules by CC-laboratories (Ambato-Ecuador). No fillers or binders were added to the cooked *Lupinus* powder.

Extraction and purification of alkaloids from L. Mutabilis

Alkaloid extraction was performed as previously described.[9] The amount of alkaloid present in raw *L. mutabilis* was measured as previously described.[10]

Dose of alkaloids and cooked beans of Lupinus mutabilis

In a previous study, nearly 30% of participants who received raw *L. mutabilis* (providing 3.125 mg/kg of alkaloid) presented minor side effects.[6] To avoid the previously observed side effects, the dose of alkaloids was reduced in the present study to 2.5 mg/kg of body weight. To estimate the dose of cooked *L. mutabilis,* the amount of alkaloids present in raw beans was used as a reference.

Anthropometric measurements

A standardized clinic weight scale that also measured height was used to determine both height and weight of each participant. [11]

Blood samples

Three blood samples at 0, 60 and 90 minutes were obtained as previously described.[6]

Statistics

Descriptive statistics were calculated. Although data on glucose and insulin was not normally distributed we are presenting parametric statistics because the results and conclusions are quite similar when using both type of tests. ANOVA and Kruskal Wallis were used to test statistical significance for differences between groups. Paired t-test and Paired Wilcoxon Signed Ranks Test were used to test for differences within the treatment groups.

Results

Demographics and general characteristics of volunteers

Data indicated that there were not statistically significant differences within the treatment groups in the

Table I
Anthropometric and demographic characteristics of participants

Variable	*Alkaloid (n = 10)*	*Cooked* Lupinus *(n = 20)*	*p-value*	*Total (n = 30)*
Age	63.8 ± 10.6	59.8 ±10.3	0.329	61.13 ± 1 0.39
Males	2/4 (50.0)	2/4 (50.0)	0.584	4/4 (100.0)
Females	8/26 (30.8)	18/26 (69.2)		26/26 (100.0)
Weight	61.1 ± 10.0	74.8 ± 14.7	0.013	70.2 ± 14.7
Height	1.47 ± 0.1	1.51 ± 0.1	0.144	1.5 ± 0.1
WC	103.0 ± 31.4	106.8 ± 22.5	0.705	105.5 ± 25.3
BMI	28.2 ± 3.6	32.7 ± 5.8	0.033	31.2 ± 5.5

Table II
Comparison of blood glucose and insulin concentrations in volunteers with type 2 diabetes treated with cooked lupinus mutabilis *or its purified alkaloids*

Treatment	*Glucose 0*	*Glucose 60*	*Glucose 90*	*Difference 0 vs 60*	*p-value 0 vs 60*	*Difference 0 vs 90*	*p-value 0 vs 90*
Alkaloid	112.8 ± 18.2	112.9 ± 16.4	101.6 ± 12.6	+0.1 (+0.09%)	0.796	-11.2 (-9.9%)	0.015
Lupinus	114.4 ± 27.2	106.6 ± 25.1	98.1 ± 21.6	-7.8 (-6.82%)	0.000	-16.3 (-14.2%)	0.000
Treatment	*Insulin 0*	*Insulin 60*	*Insulin 90*	*Difference 0 vs 60*	*p-value 0 vs 60*	*Difference 0 vs 90*	*p-value 0 vs 90*
Alkaloid	9.5 ± 5.0	7.8 ± 3.4	9.1 ± 5.4	-1.7 (-8.0%)	0.083	-0.4 (-4.21%)	0.800
Lupinus	11.7 ± 6.5	11.8 ± 6.5	11.4 ± 6.3	+0.1 (+0.85%)	0.731	-0.3 (-2.5%)	0.876

demographic/anthropometric variables except weight and body mass index (BMI), which were higher in the cooked *L. mutabilis* treatment group (table I).

Effect of Lupinus mutabilis on blood glucose and insulin in patients with diabetes

Volunteers for this study were recently diagnosed patients with type-2 diabetes that were receiving 500-1.000 mg daily of metformin. Volunteers were asked to skip the morning dose the day of the study and to come to the clinic at 8:00 am, in a fasting state. Thirty volunteers were randomly allocated to consume cooked *L. mutabilis* (N = 20) or alkaloids from *L. mutabilis* (N = 10) and were treated as described above.

None of the comparisons in serum glucose or insulin concentrations between the treatment groups were statistically significant at 0, 60 or 90 minutes. However, the percentage of decrease in glucose concentration from base line to 90 minutes was higher in the cooked *Lupinus* group compared with the alkaloid group, 14.25% and 9.93% respectively (table II). On the other hand, the percentage of insulin decrease in the alkaloid treatment group at 90 min was higher than the decrease observed in the cooked *Lupinus* treatment group, 4.21% *versus* 2.56% respectively.

Comparison within treatment groups showed variable changes in serum glucose and insulin concentrations. Subjects that received alkaloids did not have significant changes in glucose levels at 60 minutes, but did have a significant decrease in glucose concentrations at 90 minutes post-treatment, a 9.9 % decrease table II. Subjects that received cooked *L. mutabilis* had significant decrease in serum glucose concentrations at 60 and 90 minutes after treatment (table II). Regarding serum insulin concentrations, there were no statistically significant changes within the groups. However insulin concentrations decreased at 60 and 90 minutes in the alkaloid group, whereas in the cooked *Lupinus* group, insulin had a small increase at 60 minutes and decreased at 90 minutes. Overall there was a decrease in insulin concentrations by 90 minutes in both treatment groups, although differences were not statistically significant Table 2. None of the patients that received cooked *L. mutabilis* or alkaloids from *L. mutabilis* experienced any side effects.

Discussion

The present study indicates that consumption of cooked *L. mutabilis* or its purified alkaloids by individuals recently diagnosed with diabetes, showed decreased blood glucose and insulin levels. The decreases in serum glucose concentrations from base line to 90 minutes after treatment were statistically significant within each treatment group. However, there were no differences between the groups. Serum insulin levels were also decreased in both treatment groups, however, the differences were not statistically significant. None of the volunteers in either treatment group presented side effects after treatments.

In a recent report it was shown that consumption of raw *L. mutabilis* powder obtained from beans directly from the plant without any processing by individuals with glucose abnormalities significantly decreased blood glucose and insulin levels.[6] The present study complements those observations, since it assessed the effect of purified alkaloid and cooked *L. mutabilis* without alkaloids.

Considering the previous report on raw *L. mutabilis* along with the present data, it is evident that the greatest decrease in serum glucose was observed in those individuals that consumed cooked *L. mutabilis* (14.25%) while the decrease after alkaloid and raw *Lupinus* consumption was 9.93% and 5.6% respectively (fig. 1A). This comparison has limitations because both studies were carried out with different populations of volunteers and at different times. The study population who received raw *L. mutabilis* was people with glucose abnormalities that were not taking any medication while in the current study participants were volunteers who were recently diagnosed with diabetes and were under treatment with low doses of metformin. The mechanism of action of metformin includes decreased hepatic glucose production, decreased intestinal absorption of glucose and improved insulin sensitivity, leading to increased peripheral glucose uptake and use.[12] Presumably the cellular changes on insulin function and use of glucose produced by metformin treatment in the volunteers with diabetes could have enhanced the changes in glucose levels seen by the consumption of cooked *L. mutabilis* or alkaloids in this study. Contrary to the statistically significant decrease in serum insulin observed after raw *L. mutabilis* consumption in our previous report, there were not significant changes after cooked *L. mutabilis* or alkaloids intake in the present study (fig. 1B). It is important to note that basal insulin concentration in our previous study was greater than that found in this study (fig. 1B). An important mode of action of metformin in Non-Insulin-Dependent Diabetes Mellitus treatment is the improvement of insulin sensitivity, reduction of insulin resistance, and reduction of insulin plasma concentrations.[13] The normal low plasma insulin levels observed at the beginning of this study could be the consequence of metformin treatment in these patients. Consequently the improvement in insulin levels after the consumption of cooked *L. mutabilis* or alkaloids was not as strong as in our previous study.

Several studies indicate that consumption of beans of the genus *Lupinus* or its derived alkaloids have positive effects on hyperglycemia and insulin release. The possible components of the beans and their mechanism of action have now started to be elucidated. Bertoglio et al. have reported the hypoglycemic effect of *Lupinus albus* seed γ-conglutin-enriched preparation during a glucose overload in rats and humans.[14] That study showed a dose response hypoglycemic effect of a γ-conglutin enriched preparation during a glucose challenge of 2 g/kg in rats. Rats received doses of the γ-conglutin preparation ranging from 50 to 200 mg/kg of body weight. The effect of the highest dose of γ-conglutin was very similar to the hypoglycemic effect of the positive control metformin (50 mg/kg body weight). In a similar manner, a progressive hypoglycemic effect was observed in 15 human volunteers that were challenged with 75 g of carbohydrate and that were treated with increasing doses of the γ-conglutin enriched preparation (ranging from 750 to 3,000 mg).[14] In that study, no effects on serum insulin concentrations were observed.[14] The chronic use of γ-congluting treatment in hyperglycemic induced rats also showed a positive effect on glucose and insulin concentrations. The concomitant treatment of glucose (overage intake

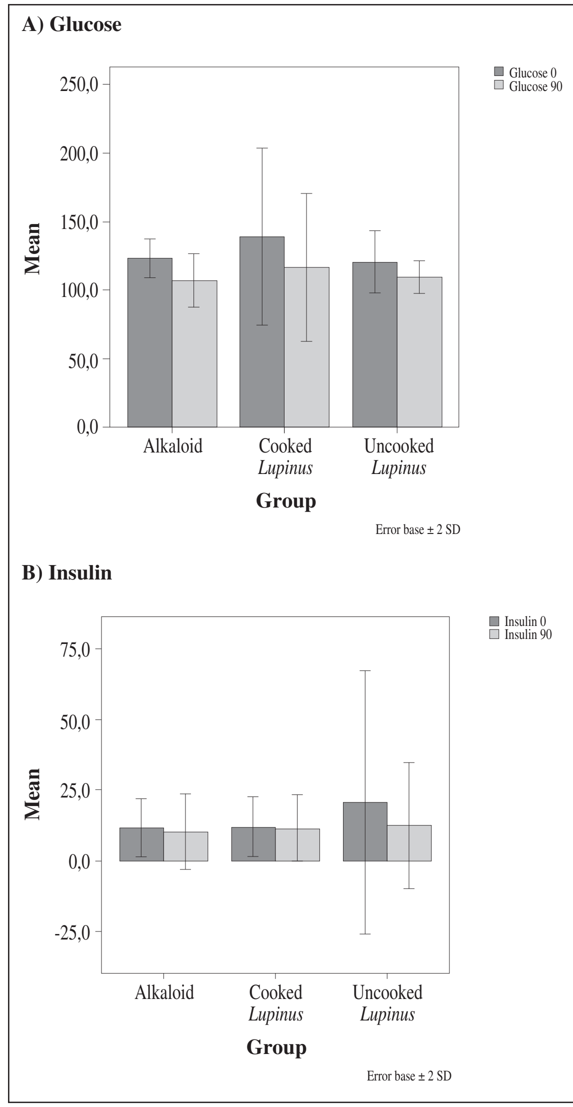

Fig. 1.—Levels of Blood Glucose (A) and Insulin (B) in Volunteers Treated with Lupinus Mutabilis: Raw, Cooked, or its Purified Alkaloids.

2-3 g/d) and γ-conglutin (28 mg/kg body weight) attenuated the increase in plasma glucose and insulin concentrations.[15] Chronic treatment with γ-conglutin also improved the state of insulin resistance as determined by the decrease of HOMAS in the treated animals.[15] Current results from this study show that consumption of cooked *L. mutabilis* beans without alkaloids decreased blood glucose. Although the presence of γ-congluting in *L. mutabilis* beans has not been determined, it is reasonable to speculate that the hypoglycemic effect observed in this study could be due to γ-conglutin or other similar components present in *L. mutabilis* beans.

Capraro J et al studying the susceptibility of γ-conglutin to proteolitic enzymes in vitro have shown that the protein is resistant to proteolysis at pH greater than 4.[16] The integrity or partial integrity of the protein could favour its absorption by intestinal epithelial cells in the upper gastrointestinal tract. γ-conglutin access to the blood stream would facilitate its systemic distribution and putative effects at the cellular level. In this context it has been demonstrated that γ-conglutin shares a mimetic action with insulin.[17] Terruzi et al. have shown that γ-conglutin stimulation of mouse C2C12 myoblastic cells elicited the activation of intracellular kinases very similar to the effects provoked by insulin. γ-conglutin stimulated the IRS-1/PI-3-kinase pathway which is critical in glucose homeostasis and protein synthesis.[17] γ-conglutin stimulation resulted in the translocation of the glucose transporter GLU-4 to the surface of the cell and the expression of myosin heavy chain.[17] Current results contribute to clarify the mechanism of action of lupin and lupin-derived alkaloids. Thus, lupin effects were observed in subjects with diabetes under basal conditions after approximately 12 hours of fasting. By this time the main source of plasma glucose is through the process of gluconeogenesis in liver and kidneys. The observed decrease in glucose levels could be the result of a direct effect of lupin or alkaloids on cells undergoing gluconeogenesis like the hepatocyte. Whether γ-conglutin, alkaloids or both contribute to stop gluconeogenesis in the liver has not been elucidated.

Alkaloids from Lupinus also have important effects on glucose and insulin homeostasis. Clinical studies have shown that intravenous administration of alkaloids from *Lupinus spp* decreases blood glucose and increases insulin in healthy volunteers or with individuals with type-2 diabetes.[18,19] The present study indicates that oral administration of alkaloids from Lupinus also decreases blood glucose without affecting significantly insulin levels. Taken together, data indicate that intravenous and the more physiological oral administration of alkaloids decreases plasma glucose levels.

No side effects were associated with cooked *L. mutabilis* or purified alkaloids from *L. mutabilis*. The lower doses of purified alkaloids used in the present study may also explain the absence of side effects. Cooked debittered *L. mutailis* is a popular traditional and affordable food in the Andean region including Ecuador. *L. mutailis* beans are an important source of protein and other important macro- and micro- nutrients and its consumption is favored as a snack or as an ingredient in several traditional dishes in Ecuador.

It would be important to determine the specific components in cooked *L. mutabilis* beans and purified alkaloids associated with beneficial effects on glucose and insulin levels in patients with diabetes. *L mutabilis* is rich in complex oligosaccharides, fiber, and omega-three fatty acids. Also, the long-term effects of these compounds in the maintenance of normal glucose and insulin levels in diabetes should be evaluated.

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