Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

The Hypoglycaemic Effect Of Pumpkin Seeds, Trigonelline (TRG), Nicotinic Acid (NA) and D-Chiro-inositol (DCI) In Controlling Glycaemic Levels In Diabetes Mellitus

Gary G. Adams PhD MRSC [a d], Shahwar Imran MSc [a d], Sheng Wang MSc [a d], Abubaker Mohammad MSc [a d], M. Samil Kok PhD [b d], David A. Gray PhD [c], Guy A. Channell PhD [c] & Stephen E. Harding PhD [d]

[a] University of Nottingham, Faculty of Medicine and Health Sciences

[b] Department of Food Engineering , Abant Izzet Baysal University , Bolu , Turkey

[c] University of Nottingham, School of Biosciences, Food Sciences , Sutton Bonington , LE12 5RD , UK

[d] University of Nottingham, School of Biosciences, National Centre Macromolecular Hydrodynamics , Sutton Bonington , LE12 5RD , UK

Accepted author version posted online: 14 May 2013.

To cite this article: Gary G. Adams PhD MRSC , Shahwar Imran MSc , Sheng Wang MSc , Abubaker Mohammad MSc , M. Samil Kok PhD , David A. Gray PhD , Guy A. Channell PhD & Stephen E. Harding PhD (2013): The Hypoglycaemic Effect Of Pumpkin Seeds, Trigonelline (TRG), Nicotinic Acid (NA) and D-Chiro-inositol (DCI) In Controlling Glycaemic Levels In Diabetes Mellitus, Critical Reviews in Food Science and Nutrition, DOI:10.1080/10408398.2011.635816

To link to this article: http://dx.doi.org/10.1080/10408398.2011.635816

Title: The Hypoglycaemic Effect Of Pumpkin Seeds, Trigonelline (TRG), Nicotinic Acid (NA) and D-Chiro-inositol (DCI) In Controlling Glycaemic Levels In Diabetes Mellitus

Running Title: The Hypoglycaemic Effect Of Pumpkin in diabetes mellitus

Gary G. Adams, PhD MRSC[1/4]✉, Shahwar Imran, MSc[1/4], Sheng Wang, MSc[1/4], Abubaker Mohammad, MSc[1/4], M.Samil Kok, PhD[2/4], David A. Gray, PhD[3], Guy A. Channell, PhD[3] and Stephen E. Harding, PhD[4]

[1]*University of Nottingham, Faculty of Medicine and Health Sciences*

[2]*Department of Food Engineering, Abant Izzet Baysal University, Bolu, Turkey;*

[3]*University of Nottingham, School of Biosciences, Food Sciences, Sutton Bonington, LE12 5RD UK;*

[4]*University of Nottingham, School of Biosciences, National Centre Macromolecular Hydrodynamics, Sutton Bonington, LE12 5RD UK*

Abstract

In the contemporary society, diabetes mellitus is considered as a common, growing, serious, costly, and potentially preventable public health problem. It is forecasted that in the year of 2030, the number of people with diabetes will go up from 117 million in 2000 to 366 million in 2030. The prevalence of diabetes will place a huge burden on health and financial structures of countries, and these will impact on individuals, as well as families and nations. Polysaccharides, para-aminobenzoic acid, fixed oils, sterol, proteins and peptides are biologically active ingredients, which are found in pumpkins. The chemicals within pumpkins such as the fruit pulp, oil from ungerminated seeds and protein from germinated seeds have hypoglycaemic properties. Preliminary investigation showed that pumpkin seeds, and the macromolecules, therein, such as Trigonelline (TRG), Nicotinic acid (NA) and D-chiro-inositol (DCI) possess hypoglycaemic properties and could assist in maintaining glycaemic control

Keywords: Hypoglycaemia; pumpkin seeds, trigonelline; nicotinic acid; d-chiroinositol

Running header (shortened title): The Hypoglycaemic Effect Of Pumpkin Seeds, Trigonelline, Nicotinic Acid and d-chiroinositol

Corresponding Author

[1]*Dr Gary G. Adams PhD MRSC*

Insulin and Diabetes Experimental Research (IDER) Group, University of Nottingham, Faculty of Medicine and Health Science, Clifton Boulevard, Nottingham, NG7 2RD UK

Tel: +44(0) 115-8230901

Fax: +44(0) 115-8230999

Email: Gary.adams@nottingham.ac.uk

Conflicts of Interest

None

Introduction

In diabetes mellitus (DM), insulin levels are too low to reduce glucose levels in the plasma and consequently, hyperglycaemia presents. As a result of this physiological state, DM can be a life threatening condition, which can lead to chronic disorders such as diabetic polyneuropathy [1] (peripheral neuropathy, autonomic neuropathy, proximal neuropathy and focal neuropathy), retinopathy [2] (macular oedema) and nephropathy [3]. Diabetes is mainly characterised by hyperglycaemia due to dysregulation of glucose metabolism. In patients without diabetes, blood glucose increases trigger the secretion of insulin by pancreatic islet beta cells [4]. The insulin binds to insulin receptors located on cells, and hence signals them to increase the rate of glucose uptake from the plasma into the cells. With the return of normal blood glucose levels, the amount of insulin in the blood again drops. Therefore, in the absence of insulin, blood glucose levels would rise to dangerously high levels, often resulting in death.

Type 1 diabetes or insulin-dependent diabetes mellitus (IDDM) usually develops in childhood and is characterised by the lack of insulin production. It is often categorised as autoimmune disease, whereby the immune system

produces antibodies, which attach to the beta cells in the pancreas and destroy them, thus stopping insulin production. For this reason, the patient becomes dependent on the external sources of insulin for survival [5].

Type 2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM), is the most common form of diabetes, comprising 90% of the worldwide population of diabetic patients and arises in middle aged people. Although these patients produce normal (or even high) levels of insulin in their blood and the insulin attaches normally to the receptors on cells, they exhibit a low rate of cellular uptake of glucose in response to insulin [6].

DM has been traditionally treated with regular injections of insulin(s). The glucose level of the patient is monitored and when hyperglycaemia occurs insulin is injected subcutaneously [7]. The saline solution of insulin is gradually absorbed into the bloodstream via the dermal capillaries, where it reaches its maximum activity at 2 to 3 hours following injection. Certain slow-acting formulations of insulin such as Lente insulin show an even more prolonged effect [8]. However, irrespective of insulin(s) used, and there are many insulins available, subcutaneous administration of insulin is

inconvenient and painful. Additionally, as insulin is administered in a non-physiological manner, targeting mainly extra-hepatic insulin-dependent tissues, hyperinsulinaemia may result [9].

Clinically, insulin replacement therapy consists of basal insulin, insulin supplementation and a prandial dose given to mimic endogenous insulin with the ingestion of food. The response occurs in two phases: 1) first-phase secretion and 2) a more prolonged second-phase release in to the portal system. Basal insulin profiles mimic the constant but small release of insulin regulating the breakdown of lipolysis and hepatic glucose production (HGP) [10]. Insulin supplementation corrects the hyperglycaemia arising either between or immediately prior to meals [11, 12]. With subcutaneous injections, the second-phase is never completely matched.

As a consequence, alternative methods of therapy and delivery must be considered. This paper focuses only on the hypoglycaemic effect of pumpkin seeds, trigonelline (Trg), nicotinic acid (NA) D-chiro-inositol and discusses the effect that these have on controlling glycaemic levels in patients, who present with diabetes mellitus.

Pumpkin (*Cucubitaceae*) seeds

Polysaccharides, para-aminobenzoic acid, fixed oils, sterol, proteins and peptides are biologically active ingredients, which are found in pumpkins [13, 14] in addition to the carotenoids and γ- aminobutyric acids found in the fruits [15-17]. Several phytochemicals such as polysaccharides, phenolic glycosides, 13-hydroxy-9Z, 11E-octadecatrienoic acid from the leaves of pumpkin, proteins from germinated seeds, have been isolated [18-21].

The chemicals within pumpkins, which have hypoglycaemic properties include polysaccharides from the fruit pulp, oil from ungerminated seeds and protein from germinated seeds [22-24].

The health protective value of the protein and peptides in pumpkin seeds has received considerable attention. Preliminary investigation showed that the protein from germinant pumpkin seeds can reduce blood glucose levels in alloxan-diabetic rats [21] and could potentially be used as alternatives to oral hypoglycemic agents. The authors are presently working on this.

Alloxan (2,4,5,6-tetraoxypyrimidine or 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative, which is a toxic glucose analogue and selectively destroys pancreatic insulin-producing cells when administered to animal species (Figure 1). As a consequence, insulin-dependent diabetes mellitus (Alloxan Diabetes) is induced with resultant characteristics similar to type 1 diabetes in humans.

Alloxan's selective toxicity to these beta cells occurs because it preferentially accumulates in beta cells through uptake via the glucose transporter, GLUT 2. In the presence of intracellular thiols, Alloxan generates reactive oxygen species (ROS) in a cyclic reaction with dialuric acid and beta cell toxicity is instigated by free radicals formed in this redox reaction.

In an experiment by Cai et al (2003), the protein from ungerminanted pumpkin seeds showed an additional improvement in glucose tolerance. In addition, it is also found that pumpkin seed oil can lessen the effects of diabetes by promoting hypoglycaemic activity [25, 26].

Li and Fu (2003) carried out an experiment on alloxan-induced diabetic rats to prove that ungerminated pumpkin seed oils and germinated pumpkin seed oils both have a hypoglycaemic effect. Different molecular weight proteins (3 kDa, between 3 kDa and 60 kDa, and over 60 kDA) were extracted from germinated pumpkin seeds post-germination for 4 days (Figure 2).

The second preparation was to test acute toxicity of these extracts (germinated pumpkin seeds protein and ungerminanted pumpkin seed oils). 80 rats were randomly divided into 2 groups with half male and half female: (1) Group 1 was fed the maximum of 8.7g/kg pumpkin seeds oil for each time; (2) 11.2 g/kg of pumpkin buds protein was fed to Group 2 rats and observation tests were run for 7 days. The results showed that there was no increased mortality or anatomical aberration confirming the safety and avirulence of the maximum of these doses.

Hereafter, ten (10) rats were randomly selected as: control (Group 1); the remaining 100 rats were injected with 170 mg/kg of alloxan to induce diabetes. 10 alloxan-induced diabetic rats in these 100 were left as alloxan-

only group (Group 2). The remaining 90 were divided randomly into 9 groups which were fed with different doses or pumpkin extract: 1) 20 mg/kg glibenclamide (Group 3); 2) 300 mg/kg of protein component A (Group 4); 3) 150 mg/kg of protein component A (Group 5); (4) 300 mg/kg of protein component B (Group 6); 5) 150 mg/kg) of protein component B (Group 7); 6) 300 mg/kg of protein component C (Group 8); 7) 150 mg/kg of protein component C (Group9); 8) 300 mg/kg (Group 10) and (9) 150 mg/kg pumpkin seed oil (Group 11). Administration occurred intragastrically for 10 days, after which (next day) blood glucose and insulin levels were measured. Oral glucose tolerance tests were also carried out by intragastric administration of 25g/kg glucose. The results for above tests are shown in Table 1 (Li, 2005).

After alloxan was injected, the levels of blood glucose increased significantly and insulin content accordingly decreased in Group 2 when compared with Group 1 (control group). In Group 3 (glibenclamide group), the content of insulin and regulations of blood glucose during 2 hours were significantly improved.

In terms of insulin production (μIU/ml), the groups were in the order of: Group 8>Group4>Group10>Group 6 when considering the highest dose (mg/ml) administered. In terms of insulin production (μIU/ml), the groups were in the order of: Group 9>Group 5>Group 11>Group 7 when considering the lowest dose (mg/ml) administered. This indicates that Protein Component C (PCC)>Protein Component A (PCA)>Pumpkin seed oil (PSO)>Protein Component B (PCB). The results indicate that the low dose of protein component C compares favourably with glibenclamide in improving insulin secretion.

When comparing blood glucose concentration levels (mmol/L), it can be seen that a similar trend is obtained from both high and low doses in all groups. After Timepoint 1 (0 Hours), the blood glucose at Timepoint 2 (0.5 hours) rises but thereafter decreases on each subsequent timepoint. Nevertheless, the blood glucose levels are comparable to Group 3 (glibenclamide).

To determine if the actively hypoglycaemic properties were present in protein seeds before germination or if they were generated during germination, Cai et al (2003) carried out an experiment, which involved

dehulling by naturally drying the fresh pumpkin seeds and removing the coats. They were then infrared dried to a constant weight, ground and extracted using supercritical fluid and an extraction device HA121-50-20.

Cai et al investigated the hypoglycaemic action in diabetic rats by soluble polysaccharides (A), protein components with molecular weights above 60 kD (B), 3-60 kD(C), and below 3 kD (D) of some pumpkin seeds, arginine (E) and pumpkin seed oil (F). Ten rats were randomly selected as control, while the other 150 rats were injected with alloxan 170 mg/kg to induce diabetes. The diabetic rats were divided into 15 groups: the model, A (1000,500 mg/kg); B (300,150 mg/kg); C (300,150 mg/kg); D (300,150 mg/kg); E (100,50 mg/kg); F (300,150 mg/kg) and Gilbenclamide (20mg/kg). These above components were intragastrically administered for 10 days and then blood samples were taken to determine serum glucose and insulin, and also to test for glucose tolerance. Results indicated in diabetic rats, that the blood insulin level was significantly increased by the soluble polysaccharides, the protein components with molecular weight over 60 kD and below 3 kD after 4 days germination. The blood glucose tolerance was improved by seed oil, protein components with molecular weights 3-60 kD

and arginine. In conclusion, Cai et al established that the protein components with molecular weights about 3-60 kD had the best hypoglycaemic action, in terms of improving the blood glucose tolerance and decreasing the blood glucose level of diabetic rats.

Trigonelline and nicotinic acid

Beside polysaccharide macromolecules, there are still other compounds contained in pumpkin, which express anti-diabetic effects, especially those compounds with low molecular weight (Yoshinari, 2009). In order to prove the hypoglycaemic effect of Trigonelline (TRG) and nicotinic acid (NA) in pumpkin (Figure 3), Yoshinari (2009) fed TRG and NA in a diet to GK rats.

In the first of these experiments (Experiment 1) and after acclimatizing for 3 days, the GK rats were assigned 2 groups of 5, each fed on either the basal diet (control) or the basal group (pumpkin). In experiment 2, and after acclimatizing for 4 days, the GK rats were assigned to 3 groups of 6 each, fed on a basal diet (control) or the basal diet containing trigonelline (TRG) or the nicotinic acid (NA group). 5 Wistar rats were fed on the basal diet

(normal rats) without diabetes. Equimolar amounts of 0.406 mM of TRG and NA were included in the diet.

With respect to experiment 1, the pumpkin effects on the blood glucose tolerance determined post-feeding of the experimental diet for 49 days (Figure 4A). 30 minutes after glucose loading, the results indicated that the blood glucose level, was significantly lower in the pumpkin group when compared to the control. The area under the curve (AUC) for the oral glucose tolerance test was also significantly lower for the pumpkin as compared to the control Figure 4B.

With regards to experiment 2, the fasting blood glucose levels did not differ amongst the controls (CON), TRG and NA groups. However, the fed blood glucose levels post-3 and 5 weeks tended to be lower and significantly lower in the TRG group than in the CON group. The fed glucose level in the NA group tended to be lower only post-5 weeks (Figure 5A/B).

The TRG and NA effects on blood glucose tolerance are shown in Figure 5C. After 15 and 30 minutes post-glucose loading, the blood glucose levels in the

TRG and NA groups did not differ from the control. Those in the TRG group, however, were significantly lower than the control and NA 60 and 120 minutes post-loading. The AUC in OGTT is indicated in Figure 5D. The TRG group was also significantly lower than the control and NA groups, although the AUC of the NA group did not differ from that of the control. Changes in the serum insulin during OGTT are indicated in Figure 5E.

Figure 6 represents the HbA1c levels measured post-1, 4 and 6 weeks of the feeding programme of dietary trigonelline and nicotinic acid. Although the HbA1c levels after weeks 1 and 4 did not differ amongst the GK rats, the levels post-6 weeks were significantly lower in the TRG and NA groups compared to the control.

D-chiro-inositol

D-chiro-inositol (DCI) is a chemical variant of inositol, a B vitamin. It is a member of a family often referred to as "inositol," (Figure 7) although it encompasses several isomers and is reported to be an important secondary messenger in insulin signal transduction [27].

DCI has classically been found in plants and insects. A rich source in plants is pinitol, the 3-0-methyl ether extracted from pine wood [28]. DCI can also be found in significant quantities in buckwheat farinetta, and some other foods (Soy Lecithin, Fig Leaf Melon, Legumes) but not abundant in most diets. It is possible that in higher vertebrates DCI is made from myo-inositol via the action of an epimerase [29].

The biological significance of DCI is that it accelerates the dephosphorylation of glycogen synthase and pyruvate dehydrogenase which are rate-limiting enzymes of non-oxidative and oxidative glucose disposal [27].

A malfunction in the epimerization of myo-inositol to chiro-inositol in the insulin sensitive tissues of the GK type 2 diabetic rats has been reported. It is possible that, DCI may act to bypass a defective normal epimerization of myo-inositol to D-chiro-inositol, which is associated with insulin resistance and could partially restore insulin sensitivity and glucose disposal. In an experimental application of DCI to diabetic rats, Rhesus monkeys and humans, it was found to accelerate glucose disposal and sensitize insulin action [27].

A study by Kennington et al, (1990) confirmed the increased myo-inositol excretion in type 2 diabetics compared to controls and a decreased excretion of chiro-inositol [30]. This was also reported by Suzuki et al (1991) in the urine of GK rats, a non-obese type 2 diabetic model, developed by inbreeding rats selectively for insulin resistance [31].

Another study on the insulin sensitivity showed that a decreased urine chiro-inositol excretion rate is related linearly to decreased insulin sensitivity (ie increased insulin resistance) [32]. Similar correlations have been obtained in humans comparing controls with glucose intolerant nondiabetics and type 2 diabetics [33]. Thus decreased urine chiro-inositol as well as increased myo-inositol may be used as a measure of insulin resistance.

DCI also appears to have substantial beneficial effects for polycystic ovary syndrome (PCOS), an observation rationalized by the apparent role of DCI in the etiology of PCOS [34, 35]. In double-blind studies, women with PCOS who received DCI experienced the following statistically significant benefits when compared with a control group: lowered free and total testosterone,

lowered blood pressure, increased insulin sensitivity and a corresponding improvement in glucose disposal, and increased frequency of ovulation [36].

Conclusion

The symptoms of diabetes itself are not serious, but other severe pathological and functional changes occur as a result of the complications of diabetes. Until now, it is found that more than 56 types of diabetes, and subsumed within them, Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) are the most common kinds. T1DM is where insulin is fundamentally important and T2DM, where the condition is controlled with insulin and diet. To diagnosis diabetes, the most recommended tested methods presently are fasting plasma glucose, 2-hour post-load plasma glucose and hemoglobin A_{1c} (HbA1c) tests.

Presently, there are many different types of insulins available for clinical use but these are not always suitable for it's users and, in some instances, have problematic side effects. Therefore, alternative approaches to the prevention and treatment of DM are required. The recent application of

herbal preparations and dietary plants, in the treatment of DM, have been in the spotlight. As one of dietary plants, pumpkin and its active hypoglycaemic properties are widely studied. The hypoglycaemic effect of polysaccharides within pumpkin and other macromolecules such as trigonelline (TRG) and nicotinic acid (NA) have shown improved properties in reducing glucose than compared to Xiaoke pill, a Chinese medicine. In addition, different extraction methods to isolate the protein-bound polysaccharide from pumpkin fruits (PBPP) have been utilized, where doses of PBPP and glibenclamide have been compared. D-chiro-inositol, a chemical variant of inositol, is a member of a family often referred to as "inositol," and is reported to be an important second messenger in insulin signal transduction

This paper confirms the hypoglycaemic nature of natural polysaccharides and other macromolecules and, although additional studies are required, pumpkins do present an alternative method for glycaemic control.

References

1. Tuncer, S., Dalkilic, N., Esen, H.H., Avunduk, M.C., *An early diagnostic tool for diabetic neuropathy: Conduction velocity distribution.* Muscle Nerve. 2011 Feb;43(2):237-44., 2011. **43**(2): p. 237-44.
2. Alajbegovic, S., Alajbegovic, A., Omerovic, J., Musanovic, A., Lacic, E., *Influence of lasting diabetes and nonadjustable glycosylated haemoglobin on occurrence of retinopathy.* Med Glas Ljek komore Zenicko-doboj kantona, 2011. **8**(1): p. 53-56.
3. Kanwar, Y.S., Sun, L., Xie, P., Liu, F.Y., Chen, S., *A Glimpse of Various Pathogenetic Mechanisms of Diabetic Nephropathy.* Annu Rev Pathol. , 2011. **28**(6): p. 395-423.
4. Adams, G.G., Uddin, A., Vives-Pi, M., Pujol-Borrell, R., James, R.F., *Characterisation of the NES2Y cell line and its use in the production of human glucose-responsive insulin producing (hGRIP) cell lines by cell-cell fusion.* Islets. 2009 Sep-Oct;1(2):117-23., 2009. **1**(2): p. 117-23.
5. Yadav, N., Morris, G., Harding, S.E., Ang, S., Adams, G.G., *Various non-injectable delivery systems for the treatment of diabetes mellitus.* Endocr Metab Immune Disord Drug Targets. , 2009. **9**(1): p. 1-13.

6. Peter, A., Fritsche, A., Stefan, N., Heni, M., Häring, H.U., Schleicher, E., *Diagnostic Value of Hemoglobin A1c for Type 2 Diabetes Mellitus in a Population at Risk.* Exp Clin Endocrinol Diabetes, 2011.

7. Adams, G., Hunter, J., Langley, J., *Is nurse-managed blood glucose control in critical care as safe and effective as the traditional sliding scale method?* Intensive Crit Care Nurs. , 2009. **25**(6): p. 294-305.

8. Colino, E., Álvarez, M.Á,, Carcavilla, A., Alonso, M., Ros, P., Barrio, R., *Insulin dose adjustment when changing from multiple daily injections to continuous subcutaneous insulin infusion in the pediatric age group.* Acta Diabetol. , 2010. **47**((Suppl 1)): p. 1-6.

9. Casimir, M., de Andrade, P.B., Gjinovci, A., Montani, J.P., Maechler, P., Dulloo, A.G., *A role for pancreatic beta-cell secretory hyperresponsiveness in catch-up growth hyperinsulinemia: Relevance to thrifty catch-up fat phenotype and risks for type 2 diabetes.* Nutr Metab (Lond). , 2011. **8**(1): p. 2.

10. Langley, J., Adams, G.G., *Insulin-based regimens decrease mortality rates in critically ill patients: A systematic review.* Diabetes/Metabolism Research and Reviews, 2007. **23**(3): p. 184-192.

11. Arafat, A.M., Möhlig, M., Weickert, M.O., Schöfl, C., Spranger, J., Pfeiffer, A.F., *Improved insulin sensitivity, preserved beta cell function and improved whole-body*

glucose metabolism after low-dose growth hormone replacement therapy in adults with severe growth hormone deficiency: a pilot study. . Diabetologia., 2010. **53**(7): p. 1304-13.

12. Adams, G.G., Hunter, J., Langley, J., *Is nurse-managed blood glucose control in critical care as safe and effective.* Intensive & Critical Care Nursing, 2009. **25** p. 294-305.
13. Kuhlmann, H., Koetter, U., Theurer, C. , *Sterol contents in medicinal pumpkin (Cucurbita pepo convar. citrullinina var. styriaca) depending on genotype and location.* Acta Horticulturae 1999. **492**: p. 175-178.
14. Appendino, G., Jakupovic, J., Belloro, E., Marchesini, A. , *Multiflorane triterpenoid esters from pumpkin. An unexpected extrafolic source of PABA.* Phytochemistry 1999. **51**: p. 1021–1026.
15. Murkovic, M., Mulleder, U., Neunteufl, H., *Carotenoid Content in Different Varieties of Pumpkins.* . J Food Composition Anal 2002. **15**: p. 633-638.
16. Gonzalez, E., Montenegro, M.A., Nazareno, M.A., Lopez de Mishima, B.A. , *Carotenoid composition and vitamin A value of an Argentinian squash (Cucurbita moschata).* . Arch Latinoam Nutr, 2001. **51**(4): p. 395-399.
17. Zhang, H., *Determination of γ -amino-butyric acid and amino acids in pumpkin.* Food Res Dev 2003. **24**(3): p. 108-109.
18. Koike, K., Li, W., Liu, L., Hata, E., Nikaido, T., *New phenolic glycosides from the seeds of Cucurbita moschata.* . Chem Pharm Bull 2005. **53**(2): p. 225-228.

19. Bang, M.H., Han, J.T., Kim, H.Y., Park, Y.D., Park, C.H., Lee, K.R., Baek, N.I. , *13-Hydroxy-9Z, 11E, 15E-octadecatrienoic acid from the leaves of Cucurbita moschata* Arch Pharm Res 2002. **25**(4): p. 438-440.

20. Xiang, D., Han, F.Y., Liang, P. , *Extraction of pumpkin polysaccharide with sodium hydroxide.* Sci Technol Food Ind 2004. **11**: p. 120-122.

21. Li, Q.H., Fu, C.L., *Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein.* Food Chem 2005. **92**(701–707).

22. Zhang, Y.J., Yao, H.Y. , *Revealing the effective ingredient in pumpkin for reducing blood sugar.* J Chin Cereals and Oils Assoc, 2002. **17**(4): p. 59-62.

23. Zhang, Y.J., Yao, H.Y., *Composition analysis of pumpkin polysaccharide and its glucatonic effect.* . J Wuxi Univ Light Ind, 2002. **21**(2): p. 173-175.

24. Xiong, X.M., *Study on extraction and separation of effective composition of pumpkin polysaccharide and its glucatonic effect.* Chin Tradit Patent Med 2000. **22**(8): p. 563-565.

25. Cai, T.Y., Li, Q.H., Yan, H., Li, N., *Study on the hypoglycemic action of pumpkin seed protein.* J Chin Inst Food Sci Technol 2003. **3**(1): p. 7-11.

26. Fu, C., Shi, H., Li, Q., , *A review on pharmacological activites and utilization technologies of pumpkin.* Plant Foods for Human Nutrition 2006. **61**: p. 73-80.

27. Larner, J., *D-chiro-inositol--its functional role in insulin action and its deficit in insulin resistance.* Int. J. Exp. Diabetes Res. , 2002. **3**(1): p. 47–60. .

28. Anderson, A.B., *Pinitol from sugar pine stump wood.* Ind. and Eng. Chem. , 1953. **45**: p. 593-596.

29. Sun, T.H., Heimark, D.B., Nguygen, T., Nadler, J.L., Larner, J. , *Both myo-inositol to chiro-inositol epimerase activities and chiro-inositol to myo-inositol ratios are decreased in tissues of GK type 2 diabetic rats compared to Wistar controls.* Biochem. Biophys. Res. Commun., 2002. **293** (3): p. 1092–8. .

30. Kennington, A.S., Hill, C.R., Craig, J., Bogardus, C., Raz, I., Ortmeyer, H.K., *Low urinary chiro-inositol excretion in noninsulin-dependent diabetes mellitus.* N. Eng. J. Med. , 1990. **323**: p. 373-378.

31. Suzuki, S., Taneda, Y., Hirai, S., Abe, S., Sasaki, A., Suzuki, K., and Toyota, T. , *Molecular mechanism of insulin resistance in spontaneous diabetic GK (Goto- Kakizaki) rats.* . New Directions in Research and Clinical Works for Obesity and Diabetes Mellitus. , ed. H. Angel. A., N. . 1991. 6.

32. Ortmeyer, H.K., Bodkin, N.L., Lilley, K., Larner, J. and Hansen, B.C. , *Chiro inositol deficiency and insulin resistance. I. Urinary excretion rate of chiro-inositol is directly associated with insulin resistance in spontaneously diabetic rhesus monkeys.* Endocrinology 1993. **132**: p. 640-645.

33. Suzuki, S., Kawasaki, H., Satoh, Y., Ohtomo, M., Hirai, M., Hirai, A., Hirai, S., Onoda, M., Matsumoto, M., Hinokio, Y., Akai, H., Craig, J., Larner, J., and Toyota, T. , *Urinary*

chiro-inositol excretion is an index marker of insulin sensitivity in Japanese type II diabetes. Diabetes Care 1994. **17**: p. 1465-1468.

34. Schmatz, D.M., Arison, B.H., Dashkevicz, M.P., Liesch, J.M., Turner, M.J. , *Identification and possible role of D-mannitol and 2-0-methyl-chiro-inositol (quebrachitol) in Eimeria tenella.* Molec. and Biochem. Parasitol. , 1988. **29**: p. 29-36.

35. Bruzik, K.S., Hakeem, A.A., and Tsai, M.D. (1994) *Are D and L- chiro-phosphoinositides substrates of phosphoinositol- specific phospholipase. C.* . Biochem., 1994. **33**: p. 8367-8374.

36. Nestler, J.E., Jakubowicz, D.J., Reamer, P., Gunn, R.D., Allan, G., *Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome.* N. Engl. J. Med. , 1999. **Nestler JE,** Jakubowicz DJ, Reamer P, Gunn RD, Allan G (1999). "Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome". N. Engl. J. Med. 340 (17): 1314–20. (17): p. 1314–20.

Table 1 The effect on blood glucose and tolerance and insulin for extract from pumpkin seeds in diabetic rat (Li et al 2005)

Group	Dose (mg/kg)	Insulin (μIU/ml)	Blood glucose and tolerance (mmol/l)			
			0 h	0.5 h	1 h	2 h

Group 1	-	109.2±19.00	3.95±1.15	7.30±0.66	5.90±0.72	6.02±1.14
Group 2	-	62.5±21.18	23.65±11.17	27.33±9.82	22.11±10.33	20.14±9.85
Group 3	20	103.4±38.73	13.84±6.42	23.11±9.27	18.13±9.01	14.00±8.56
Group 4	300	90.8±33.13	21.38±11.19	25.10±8.80	20.49±7.74	17.34±7.58
Group 5	150	95.3±32.09	21.82±10.28	24.96±9.02	20.61±8.24	17.96±8.09
Group 6	300	77.3±27.76	8.26±6.02	18.06±7.48	13.12±6.35	11.90±5.71
Group 7	150	69.8±23.54	8.91±6.73	18.39±6.71	12.87±6.10	12.12±5.54
Group 8	300	103.6±35.50	12.36±10.56	21.50±8.79	15.77±8.71	13.24±8.40
Group 9	150	104.4±43.79	14.49±9.69	24.49±6.20	15.82±6.70	12.10±7.73
Group 10	300	90.1±30.76	11.53±9.35	17.28±9.33	14.00±10.41	11.85±9.07
Group 11	150	88.6±26.34	13.74±6.33	18.35±7.50	15.57±10.96	12.52±9.75

Figures

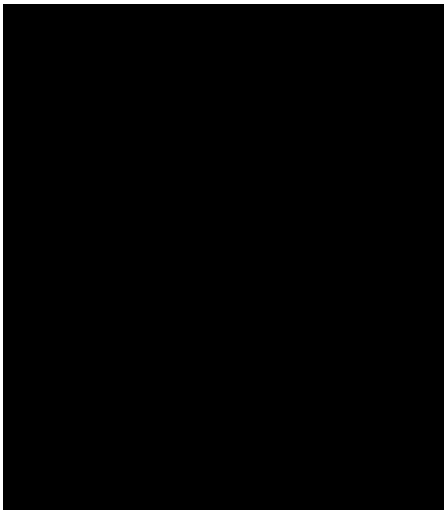


Figure 1. 2,4,5,6-tetraoxypyrimidine or 2,4,5,6-pyrimidinetetrone (Alloxan) is an oxygenated pyrimidine derivative.

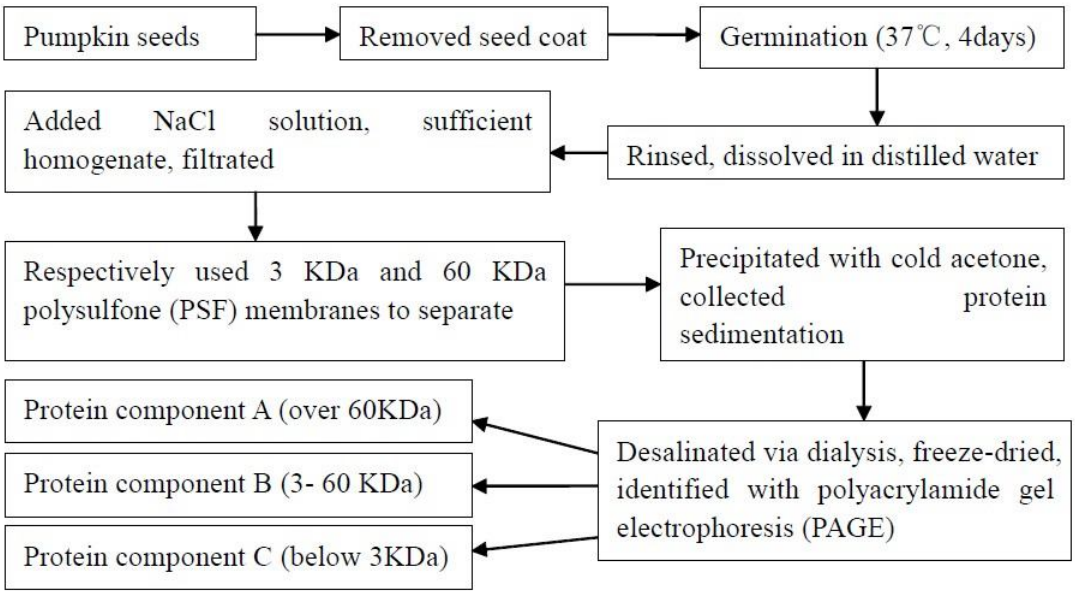
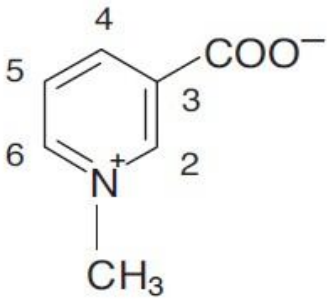
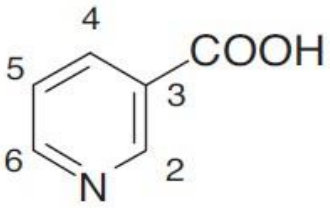


Figure 2 The process to extract protein component A, B and C from germinated pumpkin seeds (Li, 2003)



Compound I
(trigonelline)



Compound II
(nicotinic acid)

Figure 3 Chemical Structures of trigonelline and nicotinic acid

(Yoshinari, 2009)

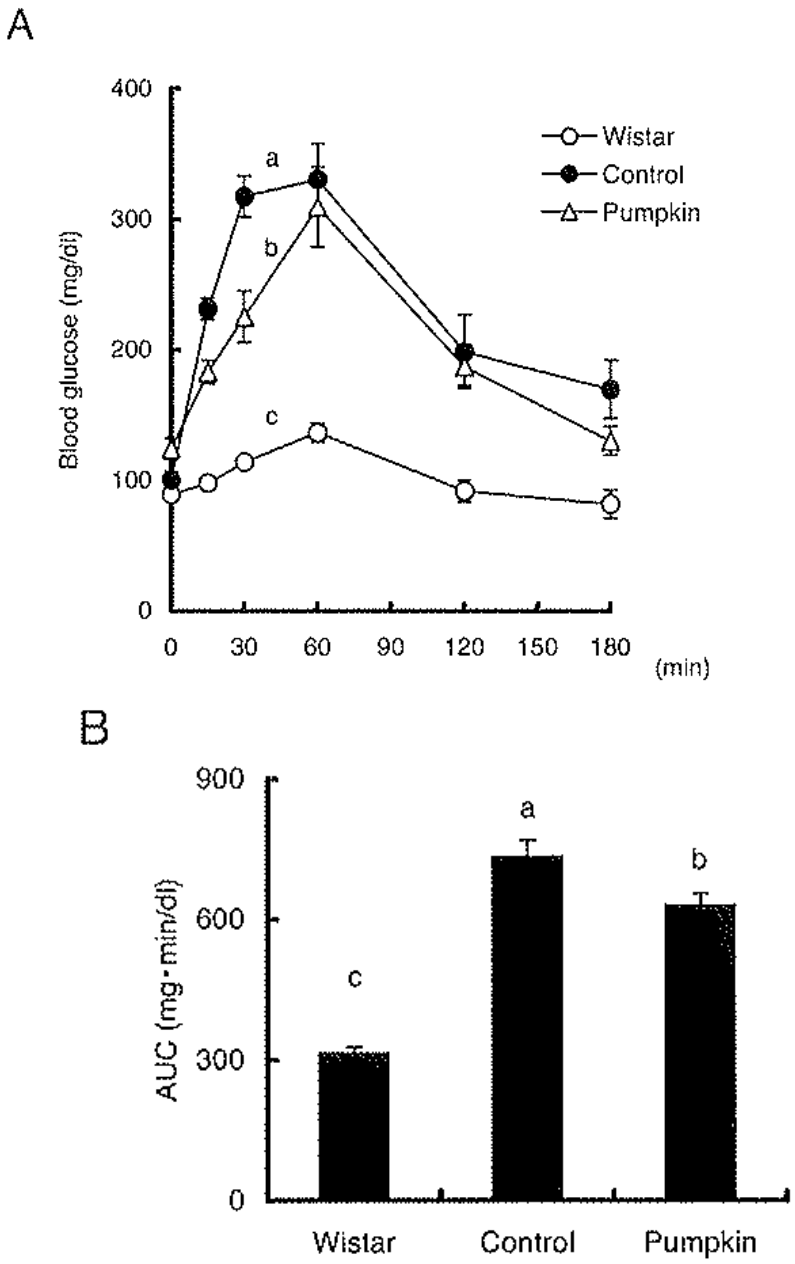
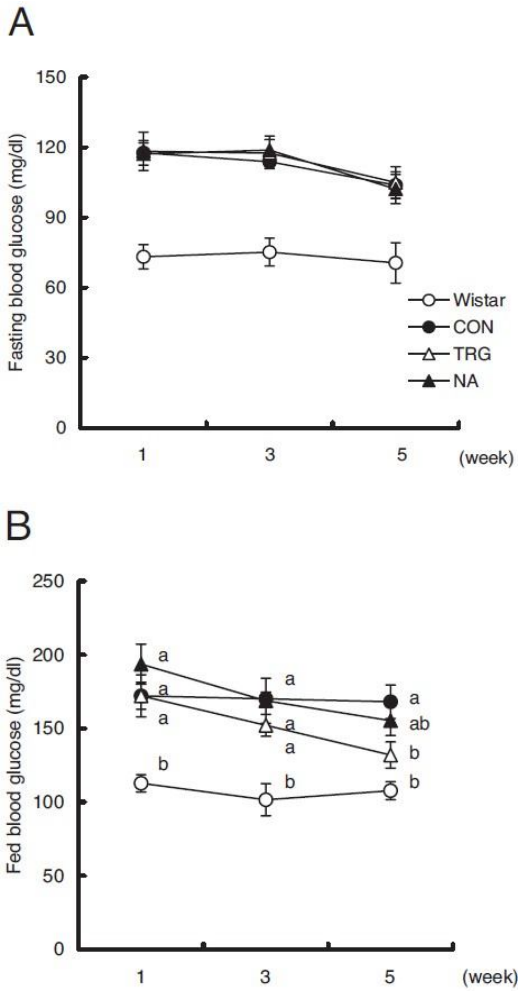
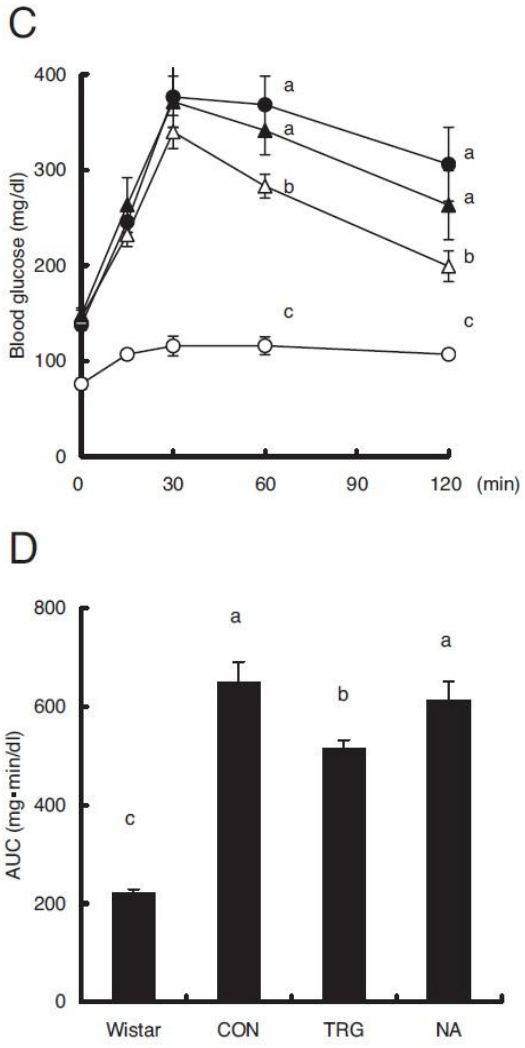


Figure 4 The effect of dietary pumpkin on the blood glucose level during the oral glucose tolerance test (OGTT). A: OGTT carried out

on day 49 of the feeding period with GK and Wistar rats fed on diet with or without pumpkin;

B:Area under curve (AUC) from OGTT. Significant values ($p<0.05$)





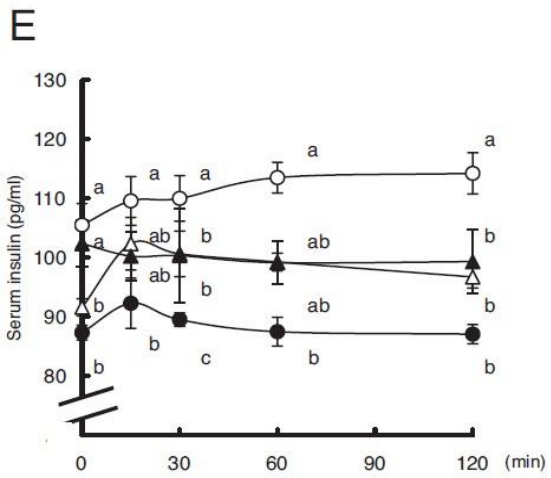


Figure 5 Effect of dietary trigonelline and nicotinic acid on the fasting (A) and fed (B) blood glucose levels, and the blood glucose level (C) and AUC (D) and insulin levels (E) during the glucose tolerance test. (Yoshinari, 2009)

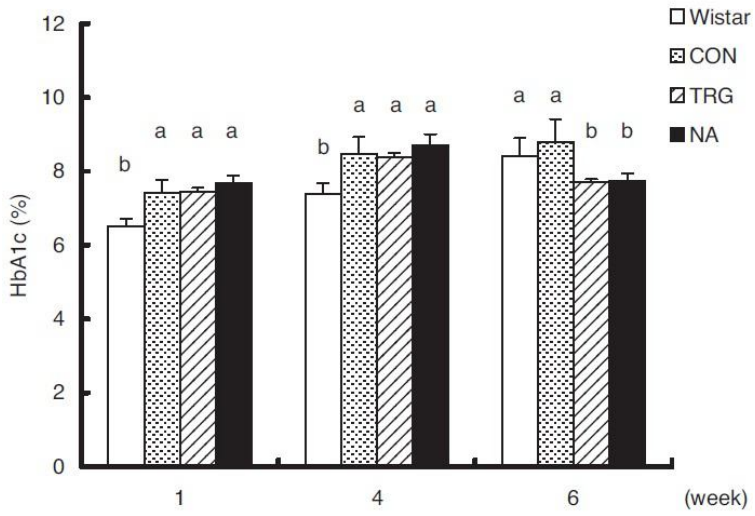
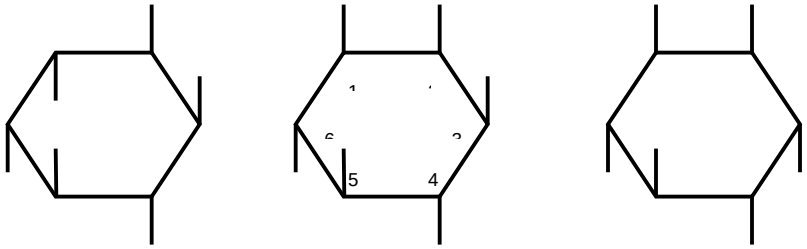


Figure 6 Effects of Dietary trigonelline and nicotinic acid on the hemoglobin A_{1c} level (Yoshinari, 2009)



L-Chiro-inositol Myo-inositol D-Chiro-inositol

Figure 7 Chemical Structures of myo-, D and L chiro-inositols. Myo-inositol is epimerized in position 1 to form L-chiro-inositol and in position 3 to form D-chiro-inositol (Larner 2002).