



Effect of Apple Cider Vinegar on Blood Glucose Level in Diabetic Mice

Maryam Iman¹, Seyed Adel Moallem^{2,3,4*}, Ahmad Barahoyee⁴

¹Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

²Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

³Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

⁴Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

ARTICLE INFO

Article Type:
Original Research

Article History:
Received: 22 December 2014
Accepted: 4 February 2015

Keywords:
Apple Cider Vinegar
Glucose
Streptozocin
Alpha Amylase
Mice
Diabetes

ABSTRACT

Background: In recent years, apple cider vinegar has been singled out as an especially helpful health remedy. It has been widely used in various dosage forms in alternative medicine for several conditions such as diabetes and obesity. In this study, the effect of apple cider vinegar on blood glucose level has been evaluated. **Methods:** Intraperitoneal (IP) injection of streptozocin 40 mg/kg/day for 4 days was used to induce diabetes in mice. The mice were divided in six groups (n=10). Two concentrations of 0.16% and 1.6% of apple cider vinegar were used in drinking water for 21 days. Normal saline and acetic acid were used as negative controls and glibenclamide by IP injection (5mg/kg) as positive control. For studying any possible combination effects, 0.16% apple cider vinegar and glibenclamide were used together. Also, the effects of apple cider vinegar on glucose tolerance test and amylase serum concentration were evaluated. **Results:** Our results indicated that apple cider vinegar in both concentrations was not effective after 3 days of the start of its administration. However, on day 7 it reduced blood glucose levels significantly and this was maintained on days 14 and 21. Glucose tolerance test showed that apple cider vinegar was effective in lowering blood glucose level after 60 minutes of glucose administration and this was maintained up to 120 minutes. Also, in both concentrations significantly reduced serum amylase levels 21 days after the start of its administration. **Conclusions:** Therefore, in this study it has been revealed that apple cider vinegar has considerable reducing effect on blood glucose levels in diabetic mice. The mechanism of this action and its significance remain to be elucidated in future investigations.

Introduction

Apple cider vinegar is an acidic solution produced by fermenting apples. It contains vitamins, minerals and many trace elements.¹ It contains a potent supply of potassium. Potassium is essential for soft tissue repair and the replacement of worn-out tissues within the body. Cider vinegar improves the health and function of the vital organs of the body by preventing excessively alkaline urine. It is a strong detoxifying and purifying agent. It breaks down fatty, mucous and phlegm deposits within the body. It also oxidizes and thins the blood, which is important in preventing high blood pressure. Cider vinegar has been found to neutralize any toxic substances that enter the body. It neutralizes harmful bacteria that may be found in certain foods, promotes digestion, assimilation and elimination.^{2,3} Toxic build-ups with the body can cause boils, blisters, acne, etc. Cider vinegar detoxifies and helps with the cleansing and clotting processes of the blood, by helping along the blood oxidation process.

When a mixture of cider vinegar and water is taken before a meal (particularly food served in restaurants or at picnics where the preparation or duration of food left uncovered and not refrigerated is questionable), it seems to prevent diarrhea or digestive upsets.^{4,5} Cider vinegar can be taken alone or used in cooking. The best method of using apple cider vinegar is in its natural liquid form. Cider vinegar is thought to be beneficial in the treatment of arthritis, asthma, nose bleeds, osteoporosis, cancer, Candida, high cholesterol, colds, constipation, muscle cramps, colitis, diabetes, diarrhea, depression, dizziness, ear discharge, eczema, fatigue, gallstones, kidney stones, hay fever, headaches, heartburn, hiccups, indigestion, insomnia, kidney and bladder problems, metabolism, nasal congestion, sore throats, stiff joints, ulcers and weight loss.⁶ Some reports showed that vinegar effect the glucose and insulin responses to a sucrose or starch load. It is near 25 years that several in vivo and in vitro studies have analysed the effect of vinegar on glucose metabolism in

healthy subjects and in subjects with diabetes mellitus.^{7,8}

One of the major worldwide health problems is diabetes. Diabetes appears to be increasing in most countries, due to increasing population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Diabetes is a metabolic disease which affects not only the glucose metabolism but also lipid and protein metabolism. Diabetes can lead to increased cardiovascular mortality, nephropathy, neuropathy and retinopathy.⁹

There are mainly two types of diabetes—Type 1 and Type 2. In Type 1 diabetes, the hormone insulin is not produced while Type 2 diabetes mellitus (T2DM) is characterized by a relative decreased sensitivity of target tissues to the action of this hormone and progressive impairment of insulin secretion. Approximately one T2DM patients are treated with oral hypoglycemic agents to stimulate insulin secretion.^{10,11} T2DM is managed through a program that consists of lifestyle modifications including appropriate diet and exercise programs and addition of oral antihyperglycemic agents.^{12,13} Although oral hypoglycemic agents (insulin) are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications.

The common side effects associated with the main classes of drugs used for the treatment of T2DM are hypoglycemia, weight gain, gastrointestinal disorders, peripheral edema and liver disease.¹⁴ The present study investigates the hypoglycemic effects of apple cider vinegar and its combination effects with antihyperglycemic agents. It is thought to be beneficial for decreasing uses of antihyperglycemic agents and their side effects.

Methods and Materials

Apple cider vinegar with 16% concentration was obtained from Septiko Co. (Mashhad, Iran) and streptozosin (Zanosar®) from Pharmacia & Upjohn (Mich, USA). Amylase kit was obtained from Zist Chimi Co. (Iran). Glibenclamide (Daroupakhsh Co, Iran) was used. Accu-chek Glucometer (Roche, Canada) was used to determine blood glucose levels. All reagents were of analytical grade.

Animals

Mice at 6-8 weeks of age, weighing 25-30 g were obtained from Avicenna Research Institute (Mashhad, Iran). The mice were housed in a standard environment at a constant temperature of 25°C under a 12-h light/dark cycle with free access to food and drinking water.

Diabetic-induced mice

Intraperitoneal (IP) injection of streptozocin 40 mg/kg/day for 4 days was used to induce diabetes in

mice. Two weeks after streptozosin injection, blood glucose levels were measured and those with fasting glucose levels above 11.1 mmol/l were included in the study.^{15,16}

Treatment regimens with apple cider vinegar

Mice could not tolerate gavage of 8% and 16% of apple cider vinegar, probably due to the high acidity of these concentrations. Various experiments showed that concentrations of 0.16% and 1.6% of apple cider vinegar in drinking water up to 21 days are well tolerated.

Experimental Protocols

Streptozocin-diabetic mice were divided randomly into 6 groups (n=10). The method of treatment was “drinking water”. Two concentrations of 0.16% and 1.6% of apple cider vinegar were used in drinking water for 21 days.¹⁷ Normal saline and acetic acid were used as negative controls and glibenclamide by IP injection (5mg/kg) as positive control. For studying any possible combination effects, 1.6% apple cider vinegar and glibenclamide were also used together. Also, the effects of apple cider vinegar on glucose tolerance test and amylase serum concentration were evaluated. The glucose levels in the plasma were determined in groups after 3, 7, 14, 21 days from drug administration. All of samples were obtained after 4h of fasting. The plasma glucose levels were expressed in mmol/l.

Blood collection for measurement of blood glucose levels

Stressful methods of blood collection can affect blood parameters such as glucose levels. To decrease stress, the mice were acclimated to the restraining device and were warmed and placed in the restraining tube. After 4 hour of fasting, a 2 mm distal section of the mouse sterilized tail is scratched using a syringe needle and gently squeezed to obtain two drops of blood, the first of which is discarded. The second drop is applied directly to the test strip to obtain blood glucose measurement. In our experience, one reading per time point was sufficient to obtain an accurate reading, however additional readings per time point were performed randomly for results validation.

Glucose tolerance test

The test is usually used to diagnose prediabetes and diabetes, insulin resistance, and sometimes reactive hypoglycaemia. The glucose is most often given orally to determine how quickly it is cleared from the blood. The test may be performed as part of a panel of tests, such as the comprehensive metabolic panel in medical practice.¹⁸

In the last day of treatment, after the fasting plasma glucose was tested, all groups received 5 g/kg of glucose by gavage. The glucose levels in the plasma were determined in groups after 30, 60 and 120 minutes of glucose administration.

Measurement of blood α -amylase

Amylase is an enzyme that its catalytic function is to hydrolyze sugar and starch. It digests polysaccharides into smaller disaccharide units, eventually converting them into monosaccharides such as glucose. α -Amylase is the major form of amylase found in humans and other mammals. It is produced by the pancreas to help digestion. An amylase test measures the amount of this enzyme in a sample of blood. In the last day of treatment, mice were sacrificed and 1.5-2 ml blood samples were obtained by cardiac Puncture. Amylase activity was determined by using a diagnostic kit. The substrate was ethylidene-p-nitrophenyl maltoheptaoside (EPS-G7). Absorbance, which is directly related to α -amylase activity, was measured at 405 nm and 37 °C using an auto analyzer (Alcyon 300® Plus, Molecular Devices Corporation, Sunnyvale, CA). Before application, the auto analyzer was calibrated with the control sera N and P (TrueLab N and TrueLab P®, respectively; Zist Chimi., Iran) and a calibrator solution (TrueCal U®, Zist Chimi Co., Iran). After calibration, the auto analyzer mixes 6 μ l of enzyme sample with 300 μ l of substrate solution, automatically and calculates the enzyme activity (IU/L).

Statistical Analysis

One-way ANOVA and t-student statistical tests were used to assess the significance of the differences. In case of significant F value, multiple comparison Tukey-Kramer tests were used to compare the means of different treatment groups. Results with $p < 0.05$ were considered to be statistically significant.

Results and discussion

Measurement of blood glucose

The effect of apple cider vinegar on glucose levels in the plasma was determined in mice after 3, 7, 14, 21 days of drug administration. Glucose blood levels in diabetic mice treated with normal saline and acetic acid were increased during 21 days (Fig. 1). Apple cider vinegar in both concentrations was not effective after 3 days of the start of its administration. However, on day 7 it reduced blood glucose levels significantly and this was maintained on days 14 and 21 ($p < 0.05$) (Fig. 1). Comparisons of treated groups showed no significant difference in their glucose levels at days 7, 14 and 21 ($p > 0.05$), indicating that apple cider vinegar at both doses has antihyperglycemic effects comparable to glibenclamide.

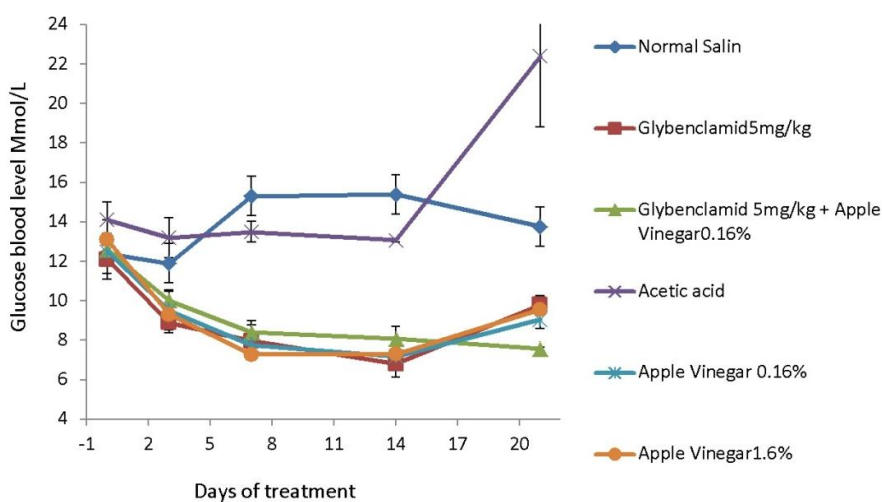


Figure 1. Glucose blood levels during 21 days.

Glucose tolerance test

In the last day of treatment after determining the fasting blood glucose, all groups received 5 g/kg of glucose by gavage. The glucose levels in the plasma were determined in groups after 30, 60 and 120 minutes. The results showed that apple cider vinegar in both concentrations significantly reduced blood glucose level after 60 minutes of glucose administration in comparison to its level after 30 minutes ($p < 0.05$) and this was maintained up to 120 minutes after the start of its consumption by diabetic mice ($p < 0.05$) (Fig. 2). This was also apparent in the positive control group.

There was no dose-dependent effect as no significant difference was observed between the two apple cider vinegar groups ($p > 0.05$).

As the glucose level did not rise significantly after glucose administration after 30 minutes in the combination treated group (apple cider vinegar + glibenclamide), there was no significant reduction in the glucose levels after 60 and 120 minutes as compared to glucose level at 30 minutes ($p > 0.05$). However, the glucose levels in this group were the lowest among all treated groups (Fig. 2).

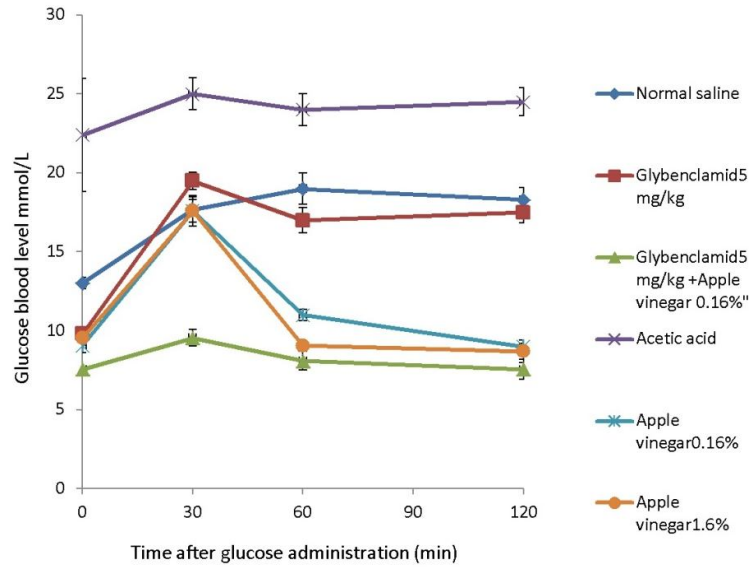


Figure 2. Glucose blood levels after administrations of 5g/kg glucose in diabetic-mice at day 21.

Measurement of blood α -amylase

In the last day of treatment, α -amylase was determined. Apple cider vinegar in both concentrations significantly reduced α -amylase levels in serum after 21 days ($p < 0.05$) (Fig. 3). The higher concentration of apple cider vinegar has more effect on reducing α -amylase serum

levels compared to the lower concentration of apple cider vinegar ($p < 0.05$). Interestingly, glibenclamide administration did not affect α -amylase serum levels alone or in combination to apple cider vinegar, compared to normal saline and apple cider vinegar, respectively (Fig. 3).

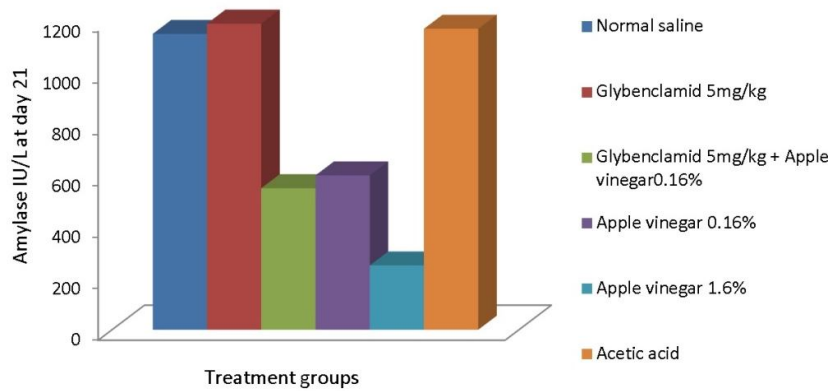


Figure 3. Amylase blood levels after 21 days of treatment.

In previous studies, it was demonstrated that vinegar affect glucose metabolism in healthy or in patients with diabetes mellitus, improves insulin sensitivity in healthy or in patients with insulin-resistant and could further delay gastric emptying, so do postprandial hypoglycaemia. But some studies showing no beneficial effect on glucose metabolism that show several factors affect vinegar effects.¹⁹⁻²¹ There is much interest in identifying diet patterns that could possibly reduce hyperglycemia. The aim of this study was to investigate the effect of apple cider vinegar on blood glucose level. Our results indicated that apple cider vinegar on days 7 and 14 reduced blood glucose levels

significantly ($p < 0.05$) and was effective in lowering blood glucose level after 60 minutes of glucose administration and this was maintained up to 120 minutes. Also, apple cider vinegar in both concentrations significantly ($p < 0.05$) reduced serum amylase levels 21 days after the start of its consumption by diabetic mice. So these results showed the favorable effects of apple cider vinegar on glucose level and it can be used in patient with diabetes type 2.

Conclusions

Our results indicated that apple cider vinegar in both concentrations was not effective after 3 days of the start

of its administration in diabetic mice. However, on day 7 it reduced blood glucose levels significantly ($p < 0.05$) and this was maintained on days 14. A slight rise in blood glucose levels was observed on day 21 that was not significant ($p < 0.05$). The findings of the present investigation showed that blood glucose levels in the acetic acid group were comparable to the normal saline group at days 3, 7 and 14. However, they were higher than the normal saline group at day 21 suggesting that continuous consumption of acetic acid probably due to its acidic property might induce stress in treated mice. This increased stress can lead to a rise of glucose blood levels. Although the cider vinegar has acidic property, probably its “therapeutic” effect in lowering glucose levels was able to overcome this property.

Glucose tolerance test showed that apple cider vinegar was effective in lowering blood glucose level after 60 minutes of glucose administration and this was maintained up to 120 minutes. Also, apple cider vinegar in both concentrations significantly ($p < 0.05$) reduced serum amylase levels 21 days after the start of its consumption by diabetic mice.

In this study it has been revealed that apple cider vinegar has considerable reducing effect on blood glucose levels in diabetic mice suggesting a useful outcome in reducing the risk diabetes due to its antihyperglycemic effect in diabetic mice.

Although the full mechanism of this effect is unclear, one probable mechanism could be the effect of apple cider vinegar α -amylase. Reduction of α -amylase in liver cells can suppress the conversion of carbohydrates (polysaccharides) into smaller saccharide units such as glucose leading to a reduction in blood glucose levels. Whether apple cider vinegar has any effect on insulin action in peripheral tissues, such as skeletal muscles and adipocytes any other probable mechanisms are unclear that can be further studied. More work is needed to determine the exact nature of the active ingredients.

Authors' contributions

MI conceived of the study and helped to draft the manuscript, SAM is head of study and participated in its design and coordination and helped to draft the manuscript. AB participated in the taking result and performed the statistical analysis.

Acknowledgements

We appreciate the technical assistance support of Mashhad University of Medical Science.

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