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Apple Cider Vinegar Attenuates Lipid Profile in Normal and Diabetic Rats

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Abstract: In this study, the effect of apple cider vinegar on Fasting Blood Glucose (FBG), glycated haemoglobin (HbA1c) and lipid profile in normal and diabetic rats was investigated. Diabetes was induced in male Wistar rats (300±30 g) by the intraperitoneal injection of streptozotocin (60 mg kg^{-1} of body weight). Both normal and diabetic animals were fed with standard animal food containing apple cider vinegar (6% w/w) for 4 weeks. Fasting blood glucose did not change, while HbA1c significantly decreased by apple cider vinegar in diabetic group (p<0.05). In normal rats fed with vinegar, significant reduction of low density lipoprotein-cholesterol (LDL-c) (p<0.005) and significant increase of high density lipoprotein-cholesterol (HDL-c) levels (p<0.005) were observed. Apple cider vinegar also reduced serum triglyceride (TG) levels (p<0.005) and increased HDL-c (p<0.005) in diabetic animals. These results indicate that apple cider vinegar improved the serum lipid profile in normal and diabetic rats by decreasing serum TG, LDL-c and increasing serum HDL-c and may be of great value in managing the diabetic complications.

Key words: Apple cider vinegar, diabetic rats, glycemic index, triglyceride, HDL-c

INTRODUCTION

Diabetes mellitus is one of the major chronic disease in all populations (Wild *et al.*, 2004). In addition to hyperglycemia, the most common feature in diabetes is dyslipidemia which is contributed to the development of atherosclerosis (Creager *et al.*, 2003). Therefore, the ideal treatment for diabetes would be an agent that controls the glycemic level and prevents the progression of atherosclerosis. The currently available agents for treatment of diabetes are expensive, not easily accessible and have several side effects (Ramesh and Pugalendi, 2005). Thus, a large number of studies are involved to find natural hypoglycemic and hypolipidemic products as alternatives to the synthetic ones.

Vinegar is produced from fermentable carbohydrate source such as apples, grapes and molasses (Johnston and Gass, 2006). It is commonly used as a food preservative and as a seasoning in different diets (O'Keefe *et al.*, 2008; Hlebowicz *et al.*, 2007). The main component of vinegar is acetic acid, being present at concentrations of 3-5% (Sakakibara *et al.*, 2007). Other constituents of vinegar include some vitamins, mineral salts, amino acids, polyphenolic compounds and organic acids (Johnston and Gass, 2006). Recently, vinegar has been reported to have some beneficial effects on postprandial blood glucose in healthy and diabetic subjects (Johnston *et al.*, 2004; Johnston and Buller, 2005; Ostman *et al.*, 2005; Leeman *et al.*, 2005). Apple cider vinegar which is made by fermentation of apple juice has been used as a folk medicine and its consumption as a home remedy for diabetes is widely advertised in mass media. However, scientific database about its biological effects is scarce. Thus, the present study was conducted to investigate the effect of apple cider vinegar on fasting blood glucose, HbA1-c and lipid profiles in healthy and diabetic rats.

MATERIALS AND METHODS

This study was conducted in July 2005 at Animal Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Islamic Republic of Iran.

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Animals: Forty male Wistar rats (300±30 g; 4-5 months old) were obtained from the animal research center (Jondyshapour Ahvaz University of Medical Sciences, Ahvaz, Iran). They were housed in the colony in cages (2-3 rats in each cage) under controlled conditions at a room temperature of 22-24°C, 55-60% humidity and a 12 h light/dark cycle. Standard animal pellets (Chavdane, Shahreza, Iran) and tap water were provided *ad libitum*. Nine rats died accidentally because of diabetes, so the provided results were from 31 rats.

Induction of diabetes: Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, United State). Diabetes was induced by intraperitoneal injection of a single dose of streptozotocin (60 mg kg^{-1} b.wt.) dissolved in normal saline (Yamamoto *et al.*, 2000). The control rats received only the same volume of saline. STZ-injected animals were allowed to drink 4.5 g L^{-1} sodium chloride solution instead of water for the whole period of treatment to prevent diuresis and were given 5% glucose solution in the 3rd day after STZ ingestion to prevent hypoglycemic mortality (Khaksari *et al.*, 2004). Diabetes was confirmed by measuring fasting blood glucose, 6 days after STZ injection. The animals with blood glucose ≥150 mg dL^{-1} were considered to be diabetic (Trivedi *et al.*, 2004).

Treatment diet: Apple cider vinegar (Yeko-Yek, Iran) was purchased from local shop containing 4.4 g dL^{-1} acetic acid. The animal pellets were powdered by a kitchen blender and mixed with apple cider vinegar (6 g vinegar/100 g animal food) (Kondo *et al.*, 2001). This mixture was then transformed into a pellet form using an industrial meat mincer. The pellets were then dried at room temperature and used for the experiment. The treatment was started on the day 8th after STZ-injection and continued for 4 weeks.

Experimental design: The animals were randomly divided into four groups of ten, classified as: normal: normal rats treated with apple cider vinegar, diabetic control: diabetic rats treated with apple cider vinegar. Blood samples (2-3 mL) were collected after 12 h fasting from the tail vein under ketamine anesthesia (90 mg kg^{-1}, i.p.), before and after the treatment period (end of the 4th week).

Serum was separated by centrifugation at 3000 x g at 4°C for 15 min and stored at -20°C for biochemical analysis. Fasting blood glucose, Total Cholesterol (TC), triglyceride and high density lipoprotein cholesterol concentrations were determined by enzymatic colorimetric assays using commercially available kits (Pars Diagnostics, Iran). Low density lipoprotein cholesterol was estimated using Freidewald formula

(Friedewald *et al.*, 1972). The concentration of HbA1-c in whole blood was measured by ion-exchange column chromatography (Hb-Gold system, Italy).

Statistical analysis: All results are presented as the Mean±SD. Statistical analysis were performed with the SPSS software (version 15 SPSS Inc, Chicago, IL). The comparisons of mean values within a group were performed using paired t-test, whereas those between the groups were assessed using independent sample t-test. The p-values of <0.05 were considered significant.

RESULTS

FBG and HbA1-c: As shown in Table 1, initial values for FBG were similar in normal rats and remained unchanged after intervention with apple cider vinegar. Diabetic rats exhibited elevated level of blood glucose as compared with normal rats (p<0.05). Although, diet containing apple cider vinegar had no significant effect on FBG, it resulted in a significant decrease in HbA1-c by 18.8% in diabetic rats (p<0.05).

Lipid profiles: The total serum cholesterol did not change in any group (Table 2, 3). In normal rats treated with apple cider vinegar, 47% reduction in LDL-c levels (p<0.005) and 34% increase in HDL-c levels (p<0.005) were observed. Significant differences in these parameters were also observed in respect to normal rats (p<0.005) (Table 2).

There was a marked elevation in serum TG concentration (1.8 fold) after STZ injection in diabetic control group (p<0.005). However, supplementation with apple cider vinegar significantly reduced the serum TG compared with control group (Table 3). Serum HDL-c was significantly reduced in diabetic control rats (compared with initial values), however treatment with vinegar resulted in a marked elevation (18%) in HDL-c in diabetic animals (p<0.05).

Table 1: Concentrations of Fasting Blood Glucose (FBG) and HbA1-c before and after 4 weeks treatment with apple cider vinegar in normal and diabetic rats

Groups	FBG (mg dL^{-1})		HbA1-c (%)	
	Before treatment	After treatment	Before treatment	After treatment
Normal (n = 8)	98±13	94±14	2.9±0.3	2.8±0.2
Normal+vinegar (n = 10)	92±15	75±24	2.9±0.3	3.2±0.4
Diabetic control (n = 6)	239±68	236±80	6.0±0.8	6.0±0.9
Diabetic+vinegar (n = 7)	249±84	200±69	6.4±0.9	5.2±0.8[ab]

Data are expressed as Mean±SD. [a]p<0.05 as compared to initial values (before). [b]p<0.05 as compared to control group. Normal+vinegar: Normal rats treated with apple cider vinegar; Diabetic+vinegar: Diabetic rats treated with apple cider vinegar

Table 2: Concentrations of serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) before and after 4 weeks treatment with apple cider vinegar in normal rats

Variables (mg dL^{-1})	Normal (n = 8)		Normal+vinegar (n = 10)	
	Before treatment	After treatment	Before treatment	After treatment
TG	33±10	34±8	31±8	27±9
TC	70±14	70±11	73±14	70±9
LDL-c	36±6	36±7	36±8	19±7[ab]
HDL-c	27±7	27±6	29±6	44±08[ab]

Data are expressed as Mean±SD. [a]p<0.005 as compared to initial values (before). [b]p<0.005 as compared to normal rats treated with apple cider vinegar (Normal+vinegar)

Table 3: Concentrations of serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) before and after 4 weeks treatment with apple cider vinegar in diabetic rats

Variables (mg dL^{-1})	Diabetic control (n = 6)		Diabetic+vinegar (n = 7)	
	Before treatment	After treatment	Before treatment	After treatment
TG	36±8	65±15[a]	39±21	28±17[b]
TC	68±5	74±8	72±15	63±13
LDL-c	25±7	36±9	37±10	26±9
HDL-c	36±3	28±4[c]	27±7	33±3[c]

Data are expressed as Mean±SD. [a]p<0.005 as compared to initial values (before). [b] p<0.005 as compared to control group. [c]p<0.05 as compared to initial values (before). Diabetic+vinegar: Diabetic rats treated with apple cider vinegar

DISCUSSION

Results from the present study indicated that feeding a diet containing apple cider vinegar has a beneficial effect on serum lipid profiles of normal and diabetic rats. HbA1-c levels were also reduced in diabetic rats. However, the fasting blood glucose concentration was not altered by apple cider vinegar. Present results regarding HbA1c is consistent with the findings by Sakakibara *et al.* (2007), who reported a significant reduction of HbA1-c by acetic acid-mixed diet in diabetic KK-A(y) mice. Johnston *et al.* (2009) was also found that daily consumption of vinegar at meal time reduced the HbA1-c values in diabetic patients. The improved HbA1-c levels by vinegar could be explained by the effect of acetic acid (its main component) in lowering the glycemic index of diet (Ostman *et al.*, 2005; Sugiyama *et al.*, 2003). It has been demonstrated that the consumption of diets with low glycemic index improve HbA1-c in subjects with type 2 diabetes (Wolever *et al.*, 1992; Brand-Miller *et al.*, 2003). These results suggest that vinegar may improve glycemic control. However, this is contrary to the result of the earlier study that showed no changes in HbA1-c after administration of white vinegar (Shishehbor *et al.*, 2007). Further studies will be required to confirm the beneficial effects of vinegar on FBG and HbA1-c.

Diabetes mellitus is associated with profound alterations in plasma lipid and lipoprotein profile which are considered as risk factors for coronary heart disease (Betteridge, 1997; Creager *et al.*, 2003). Increased plasma TG and reduced HDL-c levels are the key characteristics of dyslipidemia in type 2 diabetes (Lehto *et al.*, 1997). The current study showed that the administration of apple cider vinegar decreased the elevated plasma TG levels in diabetic rats. This is consistent with the finding by Bender *et al.* (2002), who reported that oral administration of apple cider vinegar to normal mice induced a significant reduction in plasma TG levels. Similar results were also observed in the earlier study in diabetic rats treated with white vinegar (Shishehbor *et al.*, 2007). Furthermore, Fushismi *et al.* (2006) have reported that acetic acid lowered serum TG in rats which were fed a cholesterol-rich diet. Although higher plasma TG concentrations in diabetic mice treated with acetic acid were observed by Sakakibara *et al.* (2007), while TG levels in the liver was significantly decreased. Taken together, these results indicate that ingestion of vinegar may affect plasma TG concentrations. The hypotriglyceridemic effect of apple cider vinegar might be due to the reduction of hepatic TG storage (Bender *et al.*, 2002). This could be further supported by the findings of Fushismi *et al.* (2006), who reported that dietary acetic acid reduces serum TG concentrations in rats through inhibition of lipogenesis in the liver, together with a concomitant enhancement of fatty acid β-oxidation.

The observed reduction in plasma triglyceride seen in diabetic rats was paralleled by increased plasma HDL-c concentration. Furthermore, apple cider vinegar lowered serum LDL-c and increased serum HDL-c in normal rats. These remarkable effects were also induced by white vinegar in normal rats in the earlier experiment (Shishehbor *et al.*, 2007). The possible mechanism attributed to these findings could be related to the lowering effect of vinegar/or acetic acid on the glycemic index (Ostman *et al.*, 2005; Sugiyama *et al.*, 2003). It has been found that the lower glycemic index diets are able to increase HDL-c and reduce LDL-c levels (Leeds, 2002; Slyper *et al.*, 2004). Furthermore, the low glycemic diet has been shown to decrease serum LDL-c concentrations in diabetic patients (Jarvi *et al.*, 1999). Ford and Liu (2001) also reported an inverse relation between dietary glycemic index and plasma HDL-c concentrations in adults. The results of the present study might also be due to the effect of polyphenols in apple cider vinegar (Kahle *et al.*, 2006). Apple polyphenols have been shown to decrease the serum LDL-c levels in healthy human (Nagasako-Akazome *et al.*, 2007) and increase the serum HDL-c in rats (Osada *et al.*, 2006) and in hamsters (Lam *et al.*, 2008). These changes in HDL-c and LDL-c concentrations could

be possibly contributed to the suppression of intestinal lipoprotein secretion by apple polyphenols (Vidal *et al.*, 2005). These results suggest that apple cider vinegar may improve lipoprotein pattern not only by lowering the glycemic index but also by its polyphenolic compounds.

Findings of the present study show a favorable effect of apple cider vinegar on plasma lipoprotein modification managing the diabetic complications.

ACKNOWLEDGMENTS

REFERENCES

Bender, B., Z. Kiss and L. Bardos, 2002. Effect of apple cider vinegar on plasma lipids (model experimental on mices). Proceedings of the 7th Internet World Congress for Biomedical Sciences (Abstract), Apr. 14-20.

Betteridge, J., 1997. Lipid Disorders in Diabetes Mellitus. In: Textbook of Diabetes, Pickup, J.C. and G. Williams (Eds.). Blackwell Science, London, pp: 1-55.

Brand-Miller, J.C., S. Hayne, P. Petocz and S. Colagiuri, 2003. Low glycemic index diets in the management of diabetes: A meta-analysis of randomized controlled trials. Diabetes Care, 26: 2261-2267.

Creager, M.A., T.F. Luscher, F. Cosentino and J.A. Beckman, 2003. Diabetes and vascular disease: Pathophysiology, clinical consequences and medical therapy: Part I. Circulation, 108: 1527-1532.

Ford, E.S. and S. Liu, 2001. Glycemic index and serum high-density lipoprotein cholesterol concentration among us adults. Arch. Inter. Med., 161: 572-576.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of LDL cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Fushismi, T., K. Suruga, Y. Oshima, M. Momoko, Y. Tsukamoto and T. Goda, 2006. Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. Br. J. Nutr., 95: 916-924.

Hlebowicz, J., G. Darwiche, O. Bjorgell and L. Almer, 2007. Effect of apple cider vinegar on delayed gastric emptying in patients with type 1 diabetes mellitus: A pilot study. BMC Gastroenterol., 7: 46-46.

Jarvi, A.E., B.E. Karlstrom, Y.E. Granfeldt, I.E. Bjorck, N.G. Asp and B.O.H. Vessby, 1999. Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low- glycemic diet in type 2 diabetic patients. Diabetes Care, 22: 10-18.

Johnston, C.S., C.M. Kim and A.J. Buller, 2004. Vinegar improves insulin sensitivity to a high-carbohydrate meal in subjects with insulin resistance or type to diabetes. Diabetes Care, 27: 281-282.

Johnston, C.S. and A.J. Buller, 2005. Vinegar and peanut products as complementary food to reduce postprandial glycemia. J. Am. Diet. Assoc., 105: 1939-1942.

Johnston, C.S. and C.A. Gass, 2006. Vinegar: Medicinal uses and antiglycemic effect. Med. Gen. Med., 8: 61-61.

Johnston, C.S., A.M. White and S.M. Kent, 2009. Preliminary evidence that regular vinegar ingestion favorably influences hemoglobin A1c values in individuals with type 2 diabetes mellitus. Diab. Res. Clin. Pract.

Kahle, K., M. Kraus and E. Richling, 2006. Polyphenol profiles of apple juices. Mol. Nutr. Food. Res., 49: 797-806.

Khaksari, M., M. Mahmoodi, F. Ferdosi, G. Asadi-Karam and M. Shariati, 2004. The effect of Trifluoperazine on increased vascular permeability in an experimental model of chronic diabetic rat. Physiol. Pharmacol., 9: 47-55.

Kondo, S., K. Tayama, Y. Tsukamoto, T. Nagasava, K. Ikeda and Y. Yamori, 2001. Antihypertensive effects of acetic acid and vinegar on spontaneously hypertensive rats. Biosci. Biotechnol. Biochem., 65: 2690-2694.

Lam, C.K., Z. Zhang, H. Yu, S.Y. Tsang, Y. Huang and Z.Y. Chen, 2008. Apple polyphenols inhibit plasma CETP activity and reduced the ratio of non-HDL to HDL cholesterol. Mol. Nutr. Food. Res., 52: 950-958.

Leeds, A.R., 2002. Glycemic index and heart disease. Am. J. Clin. Nutr., 76: 286-289.

Leeman, M., E. Ostman and I. Bjorck, 2005. Vinegar dressing and cold storage of potatoes lowers postprandial glycaemic and insulinaemic responses in healthy subjects. Eur. J. Clin. Nutr., 59: 1266-1271.

Lehto, S., S.M. Haffner, K. Pyorala, V. Kallio and M. Laakso, 1997. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM. Diabetes, 46: 1354-1359.

Nagasako-Akazome, Y., T. Kanda, Y. Ohtake, H. Shimasaki and T. Kobayashi, 2007. Apple polyphenols influence cholesterol metabolisminhealthy subjects with relatively high body mass index. J. Oleo. Sci., 56: 416-428.

O'Keefe, J.H., N.M. Gheewala and J.O. O'Keefe, 2008. Dietary strategies for improving post-prandial glucose, lipids, inflammation and cardiovascular health. J. Am. Coll. Cardiol., 51: 249-255.

Osada, K., T. Suzuki, Y. Kawakami, M. Senda, A. Kasai, M. Sami, Y. Ohta, T. Kanda and M. Ikeda, 2006. Dose-dependent hypocholesterolemic actions of dietary apple polyphenol in rats fed cholesterol. Lipids, 41: 133-139.

Ostman, E., Y. Granfeldt, L. Persson and I Bjorck, 2005. Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. Eur. J. Clin. Nutr., 59: 983-988.

Ramesh, B. and K.V. Pugalendi, 2005. Antihyperlipidemic and antidiabetic effects of umbelliferone in streptozotocine diabetic rats. Yale J. Biol. Med., 78: 187-194.

Sakakibara, S., T. Yamauchi, Y. Oshima, Y. Tsukamoto and T. Kadowaki, 2007. Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic kk-A(y) mice. Biochem. Biophys. Res. Commun., 334: 597-604.

Shishehbor, F., A. Mansouri, A.R. Sarkaki, M.T. Jalali and S.M. Latifi, 2007. The effect of white vinegar on fasting blood glucose, glycosylated hemoglobin and lipid profile in normal and diabetic rats. Iran. J. Endocriol. Metab., 9: 69-75.

Slyper, A., J. Jurva, J. Pleuss, R. Hoffmann and D. Gutterman, 2004. Influence of glycemic load on HDL cholesterol in youth. Am. J. Clin. Nutr., 81: 376-379.

Sugiyama, M., A.C. Tang, Y. Wakaki and W. Koyama, 2003. Glycemic index of single and mixed meal foods among common Japanese foods with white rice as a reference food. Eur. J. Clin. Nutr., 57: 743-752.

Trivedi, N.A., B. Mazumdar, J.D. Bhatt and K.G. Hemavathi, 2004. Effect of shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. Indian J. Pharmacol., 36: 373-376.

Vidal, R., S. Hernandez-Vallejo and T. Pauquai, 2005. Apple procyanidins decrease cholesterol esterification and lipoprotein secretion in Caco-2/TC7 enterocytes. J. Lipid Res., 46: 258-268.

Wild, S., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care., 27: 1047-1053.

Wolever, T., D. Jenkins, V. Vuksan, A. Jenkins, G. Wong and R. Josse, 1992. Beneficial effect of low-glycemic index diet in overweight NIDDM subjects. Diabetes Care, 15: 562-564.

Yamamoto, Y., I. Sogawa, A. Nishina, S. Saeki, N. Ichikawa and S. Iibata, 2000. Improved hypolipidemic effects of xanthan gum-galactomannan mixtures in rats. Biosci. Biotechnol. Biochim., 64: 2165-2171.