

Benefic Effect of Apple Vinegar Cider on Lipid Profile in Streptozotocin-Diabetic Rats

Ben Hmad Halima¹, Khelifi Sarra¹, Ben Jemaa Houda¹, Gara Sonia², Benzarti Anis¹, Aouidet Abdallah¹

¹ Research Unit on nutrition, regulation of metabolic systems and atherosclerosis, High School of Health Sciences, University of Tunis El Manar, Bab souika, 1007, Tunis, Tunisia.

² Laboratory of clinical biochemistry, Institute of Salah Azaiz, Bab Saadoun, Tunis, Tunisia.

ahlem2014benhmad@gmail.com

Abstract: The benefic effects of vinegar have been known for more than a century and have been demonstrated in animal as well as human studies. The purpose of the present study was to investigate the effects of apple cider vinegar on lipid profile, biomarkers of atherosclerosis and cardiovascular diseases. Male albino rats were used for the present investigation. The animals were fasted overnight and diabetes mellitus (DM) was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (65mg/kg) in citrate buffer. Vinegar cider was orally administrated to the diabetic rats. Control rats were injected with citrate buffer only and the animals were considered as diabetic, if their blood glucose values were above 250mg/dl on the third day after STZ injection. The treatment had started on the fourth day after STZ injection and this day was considered as the first day of treatment that was continued for 4 weeks. In the plasma of the animals we determined the total cholesterol (CT), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C) and triglyceride (TG) levels, alanine amino transferase (ALT) and aspartate amino transferase (AST) activities. We evaluated also CT/HDL-C, TG/HDL-C and LDL/HDL-C ratios and the atherogenic index (AI). Our treatment significantly reduced the CT values, LDL-C levels, TG levels, CT/HDL-C ratio, TG/HDL-C ratio, LDL/HDL-C ratio, AI, ALT and AST activities but increased HDL-C levels. These results indicate that apple cider improved the serum lipid profile related to the cardiovascular risk in diabetes mellitus.

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Keywords: Vinegar cider; diabetes mellitus; lipid profile

1. Introduction

Diabetes mellitus is one of the most severe problems global that is rising significantly (Anarkooli et al., 2008). Current estimates indicate that approximately 4% of the global population suffers from DM, a percentage which is expected to reach 5,4% in 2025 (Kim et al., 2006). DM is an endocrine metabolic disorder of impaired carbohydrate, fat and protein metabolism characterized by chronic hyperglycemia (Pamidi et al., 2012) which reflects deterioration in the use of glucose. This is due to defective insulin secretion or deficient to it (Bransome, 1992). It is generally recognized patients with diabetic are at risk for numerous severe complications, including diabetic obesity, hyperlipidemia and hypertension (Hamden et al., 2010; Hamden et al., 2011). Several therapeutic strategies are currently available for the treatment of this chronic metabolic disorder, including the stimulation of endogenous insulin secretion, enhancement of insulin action of the target tissues, inhibition of dietary starch and lipid degradation, and treatment with oral hypoglycemic agents (Birarri and Bhutani, 2007).

Apple vinegar cider, which possesses a characteristic aroma, taste and color, has a protective effect on certain biochemical parameters in humans as

well as animals studied. Apple cider vinegar contains pectin, trace minerals, potassium, beneficial bacteria and enzymes. Nowadays, vinegar cider is popular in folk medicine and is suggested as a remedy to various diseases, from obesity and overweight to arthritis, and also for asthma coughs, diarrhea, colitis, eczema, hair loss, and many other conditions. More conventional uses of cider vinegar are as a flavoring agent and as a food preventive (Joshi and Somesh, 2009).

This study suggests that protective agents such as apple vinegar cider may play an important role in preventing the development of diabetes, obesity and hyperlipidemia. Therefore we have investigated in this work the protective effects of apple vinegar cider on lipid profiles in serum of streptozotocin-induced diabetic rats.

2. Material and methodes

Animals

Male Albino rats (Wistar strain), weighing about 160-200 g were purchased from SIPHAT (Tunis, Tunisia). In order to prepare our treatment, the animals were kept for 2 weeks under standard conditions that were maintained of temperature at 22°C±2°C and 55% ± relative humidity with an alternating 12hour light/dark cycle. The animals were housed in a polypropylene cage and were provided with a

nutritionally standard diet (Almes, Mateur, Tunisia) and water *ad libitum*.

Diabetes mellitus was induced in overnight fasted experimental groups by a single intraperitoneal injection of STZ (Sigma chemical company, France, 65mg/kg body weight) dissolved in 0,4mM citrate buffer (pH 4.5), while the control group was injected with the citrate buffer only. Three days after administration of STZ, the tail vein blood glucose level was measured in all animals. Blood glucose levels of 250 mg/dl and above were considered diabetic (Kallem et al., 2008).

The apple cider vinegar (Vital companies, Tunisia) was orally administrated (0.51ml/Kg per day).

Animals were randomly divided into three groups (n=7 in each group): the control (C), diabetic (D) and vinegar cider diabetic (V) groups. The treatment has continued for 4weeks. Blood samples were taken for estimation of plasma lipid profiles, AST and ALT activities. The samples were centrifuged at 3000g for 10 min at 4°C and the plasma was harvested. All experiments were carried out with the approval of the local animal use committee.

Determination of ALT and AST activities, CT, HDL-C, LDL-C and TG levels

The determination of the ALT and AST activities, CT, HDL-C and TG levels was carried out by using a kit of colorimetric medicine dose provided by Eli Tech laboratories.

The LDL-C level was calculated by using the expression of Friedewald WT et al. (1972):

$$\text{LDL-C (mmol/l)} = \text{CT (mmol/l)} - \text{TG (mmol/l)} / 2.2 - \text{HDL-C}; \text{ TG} < 4 \text{ mmol/l.}$$

The atherogenic index (A.I.) was calculated by the following equations according to the methods of Benson MK and Devi K (2009).

$$\text{AI} = \text{TC} - \text{HDL-C} / \text{HDL-C.}$$

Statistical analysis

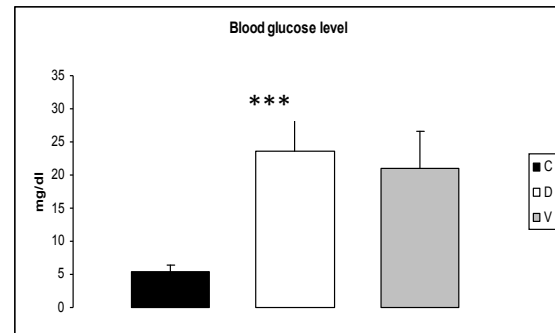
Data are presented as means \pm SEM. The determinations were performed with 5 animals per group and the differences were examined by the one-way analysis of variance (ANOVA) followed by tukey test and the significance was accepted at $p < 0.05$.

3. Results

Body weight and blood level

In our investigation, the STZ caused a significant weight loss of the diabetic rats when compared to control animals. The treatment with apple vinegar cider for 4 weeks showed a slower weight gain than normal control group. Blood glucose in control group remained normal over the whole observation. STZ led to continuously severe hyperglycemia in diabetic group but the administration of apple vinegar cider did

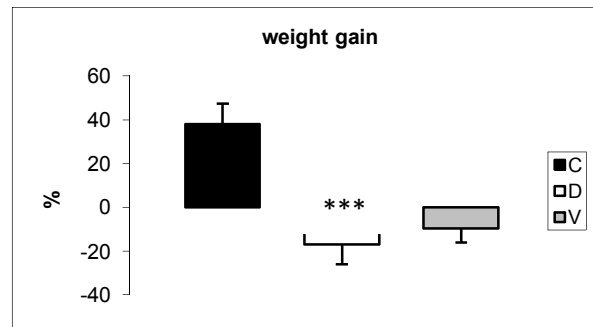
not change the fasting blood glucose (Figure 1 and Figure 2).



Values are mean \pm S.D of 7 rats in each group. *: $p < 0,05$, **: $p < 0,01$ and ***: $p < 0,001$ represent respectively the difference between (C) and (D) groups. \neq : $p < 0,05$, $\neq\neq$: $p < 0,01$ and $\neq\neq\neq$: $p < 0,001$ represent respectively the difference between (D) and (V) groups.

Figure 1. Effect of apple vinegar cider on blood glucose level in diabetic rats.

(C): Control rats; (D): STZ-diabetic rats; (V): vinegar cider diabetic rats.



Values are mean \pm S.D of 7 rats in each group. *: $p < 0,05$, **: $p < 0,01$ and ***: $p < 0,001$ represent respectively the difference between (C) and (D) groups. \neq : $p < 0,05$, $\neq\neq$: $p < 0,01$ and $\neq\neq\neq$: $p < 0,001$ represent respectively the difference between (D) and (V) groups.

Figure 2. Effect of apple vinegar cider on weight gain percentage in diabetic rats.

(C): Control rats; (D): STZ-diabetic rats; (V): vinegar cider diabetic rats.

Serum lipid profile

Serum CT, LDL-C, TG levels were significantly elevated in diabetic group in comparison to control rats. After 4 weeks of treatment with apple vinegar cider resulted a significant diminution of these parameters and levels of the levels of these parameters were resettled towards to control level. HDL-C, a

friendly lipoprotein, was decreased but not significantly. Vinegar Cider led to elevate this lipoprotein level in serum and was resettled to the control level. There was also a significant elevation in

TC/HDL, LDL/HDL, TG/HDL ratios and AI of STZ injection group in comparison to control group but administration of vinegar cider of 28 days restored these ratios and AI to near control level (Table 1).

Table 1. Effect of apple vinegar cider on plasma lipid profile.

Parameters	(C)	(D)	(V)
Total cholesterol (mg/dl)	1,56±0,16	1,96±0,15 ***	1,51±0,11 ###
LDL-C (mg/dl)	0,43±0,22	0,72±0,29*	0,45±0,22 ≠
HDL-C (mg/dl)	0,66±0,13	0,56±0,05	0,63±0,05
TG (mg/dl)	1,05±0,25	1,48±0,23 **	1,03±0,308##
Total cholesterol/HDL-C	2,40±0,41	3,50±0,58 **	2,40±0,24 ##
LDL-C/HDL-C	0,64±0,35	1,13±0,68 *	0,67±0,38 ≠
TG/HDL-C	1,66±0,58	2,60±0,31 **	1,63±0,50 ##
A.I.	1,41±0,41	2,50±0,58 **	1,40±0,24 ##

Values are mean ± S.D of 7 rats in each group. *: p<0,05, **: p<0,01 and ***: p<0,001 represent respectively the difference between (C) and (D) groups. ≠: p<0,05, ##: p<0,01 and ###: p<0,001 represent respectively the difference between (D) and (V) groups.

(C): Control rats; (D): STZ-diabetic rats; (V): vinegar cider diabetic rats.

LDL-C: low density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol, TG: triglycerides, A.I.: atherogenic index.

Transaminase activities

Table 2 shows the activities of AST and ALT. The activities of these enzymes were found to be elevated in the plasma of diabetic rats when compared to control animals. Oral administration of apple vinegar cider for 4 weeks significantly lowered the transaminase activities in STZ diabetic rats, especially the AST activity.

Table 2. Effect of apple vinegar cider on serum ALT and AST activities in diabetic rats

Enzymes	(C)	(D)	(V)
AST (UI/L)	218±33,13	278,16±60,86*	209,83±29,65##
ALT (UI/L)	92±26,67	162,71±33,10 ***	125,85±13,28≠

Values are mean ± S.D of 7 rats in each group. *: p<0,05, **: p<0,01 and ***: p<0,001 represent respectively the difference between (C) and (D) groups. ≠: p<0,05, ##: p<0,01 and ###: p<0,001 represent respectively the difference between (D) and (V) groups.

(C): Control rats; (D): STZ-diabetic rats; (V): vinegar cider diabetic rats.

ALT: alanine amino transferase; AST: aspartate amino transferase.

4. Discussion

Impact of vinegar cider on lipid profile and the coronary diseases

The cluster of lipid abnormalities associated with diabetes is defined by a high concentration of TG and small dense LDL and low concentration of HDL-C. The liver plays a central role in balancing cholesterol from all sources and regulating plasma LDL levels (Dietsch, 1993).

Assessment of risk for coronary disease is usually done by measuring TC and LDL in blood as well as the ratios TG/HDL, TC/HDL or LDL/HDL which are markers of dyslipidemia. Elevated LDL is considered to be a major risk factor, and HDL cholesterol is a major protective factor for cardiovascular diseases (Kritchevsky, 2006). The LDL/HDL ratio provides key information regarding coronary heart disease risk and is a better predictor for risk of heart disease than LDL-C alone. Several epidemiological and clinical studies have found that LDL-C/HDL-C ratio is an excellent monitor for effectiveness of lipid lowering therapies and it reflects the two way traffic of cholesterol entering and leaving the arterial intima (Fernandez, 2008).

LDL-C elevation in diabetic rats might be attributed to the reduction in the number of LDL receptor or reduced LDL binding to its receptor in these animals. Changes in hepatic LDL-receptor contribute to the elevation in blood cholesterol levels induced by diabetes and as well as to the reduction that follows hepatic cholesterol depletion.

Another risk factor for developing hyperlipidemia is the reduction in HDL-C level which attributed to its central function in the reverse of cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL

particles for further delivery to the liver for metabolism (Martinez, 2004). Moreover, increased TG and decreased HDL-C levels in diabetic rats may be attributed to decreased activity of lipoprotein lipase.

Treatment with apple vinegar cider improves lipid profile since it significantly decreases TC, TG, LDL-C levels and AI and a significant increase of serum HDL-C which became so pronounced after 4 week of treatment. These findings are in accordance with the result of Shishehbor et al (2008) who reported that apple cider vinegar administration to normal and diabetic rats improved the serum lipid profile.

Vinegar cider may improve the lipid profile by an inhibition of cholesterol production in rat liver by blocking HMG-CoA reductase, but do not impact intestinal cholesterol absorption. As a result, hepatocytes become depleted of cholesterol and respond by increasing LDL-C clearance from the blood via up regulation of hepatic LDL-C receptors and decreasing entry of LDL-C into the circulation. Treatment with apple vinegar cider might have a direct role in lipid metabolism and prevents hypercholesterolemia and hypertriglyceridemia and lowers the free fatty acids and TG levels of diabetic rats by its lipolytic activity. Vinegar may lower cholesterol levels in high fat-fed rats by inhibiting hepatic HMG-CoA reductase activity.

Vinegar may directly inhibited the lipase activity and resulted in the suppression of triglyceride digestion and thus increasing fecal elimination of fat and therefore overall effects resulted in a weight loss and improved CT, LDL and HDL-C levels.

In the other hand, Salbe et al (2009) demonstrated the antiglycemic affects of vinegar by suppressing endogenous insulin secretion. Insulin plays a central role in the regulation of lipid metabolism and has an anti-lipolytic action. Insulin resistance is believed to contribute to the atherogenic dyslipidemia by increasing the hepatic secretion of VLDL another apolipoprotein (apo) B containing lipoprotein particles as a result of increased free fatty acid flux to the liver (Krauss and Siri 2004). Insulin is a potent activator of lipoprotein lipase, promoting the catabolism of triglyceride rich lipoprotein. It not only enhances LPL activity (Brunzell et al., 1981) but also has a direct positive effect on the LPL gene, promoting LPL synthesis (Fried et al., 1993). Insulin acts also on HDL metabolism by activating LCAT and hepatic lipase activities (Ruotolo et al., 1994). It has also been shown that insulin reduces CETP activity. However, this inhibitory effect is more likely to be the consequence of the insulin- induced reduction of free fatty acids in the circulation than a direct inhibitory action on CETP (Arri et al., 1997). This may also be the result of the improvement of lipid profile in rats treated with vinegar by suppressing endogenous

insulin secretion which is an important key in the regulation of lipid metabolism.

Effect of apple vinegar cider on liver transaminase activities in diabetic rats

Moreover, the increase of the serum ALT and AST activities in the diabetic rats at the 4th week, when compared to the normal animals, is a result of the liver damage and dysfunction. It is established that the increase in the blood of ALT and AST activities is a sign of hepatic dysfunction indicating a cytolysis. Indeed, the rise in the activity of ALT is due to hepatocellular damage and is usually accompanied by a rise in AST (Mohan et al., 1989). Diabetes mellitus may induce hepatic dysfunction. The enzymes directly associated with the conversion of amino acids to keto acids are AST and ALT, and are increased in the diabetic condition. Begum and Shanmugasundaram (1978) also reported an increase in the activities of AST and ALT in the liver of diabetic animals. In addition, the increase of the liver transaminases may be attributed to hyperlipidemia resulted in injury of liver tissue so when cell membrane is damaged, these enzymes which are normally located in the cytosol, leak into the blood streams (Al-Rawi and Maisaa, 2007).

High serum cholesterol level can cause liver damages (Bolkent, 2004) and treating for 4 weeks with vinegar cider caused amelioration in the activity of these enzymes. Elevated serum ALT and AST usually indicate hepatocyte damage and the most common presentation is fatty liver. AST and ALT were increased notably in diabetic rats, and 4 weeks of apple vinegar cider treatment significantly decreased their levels suggesting that vinegar may play an important role in improving liver function.

The findings of this study demonstrate the protective effects of apple vinegar cider on lipid profile, biomarkers of the risk for coronary diseases in diabetic rats. The related protective mechanisms of apple vinegar cider have to be elucidated and a further study on the active compounds has to be carried out.

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Corresponding Author:

Ben Hmad Halima

Research Unit on nutrition, regulation of metabolic systems and atherosclerosis

High School of Health Sciences, B.P. 176, Bab souika, 1007, Tunis, Tunisia.

E-mail: ahlem2014benhmad@gmail.com.

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