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Research Article
Antihyperglycemic, Antihyperlipidemic and Modulatory Effects of Apple Cider Vinegar on Digestive Enzymes in Experimental Diabetic Rats

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Abstract

Background: Apple Cider Vinegar (ACV) is an ancient folk remedy and it is common for patients with diabetes daily because of its positive effect on blood glucose and insulin sensitivity. The present study was undertaken to investigate the possible antihyperglycemic and antihyperlipidemic effects of ACV, particularly in terme of its inhibitory effects on some carbohydrate metabolising enzymes in the intestine and the livers in normal and diabetic rats. **Materials and Methods:** The assays of the present study were conducted on adult male Wistar rats. The animals were fasted overnight and diabetes mellitus was induced by an intraperitoneal injection of freshly prepared streptozotocin (STZ). Control rats were injected with citrate buffer only. The ACV was administrated orally during 4 weeks. **Results:** Our findings indicated that the admnistration of ACV significantly decreased intestinal maltase, sucrase and lactase and hepatic glucokinase (GK) activities which led to a significant decrease in blood glucose rate and an increase in hepatic glycogen levels. In addition to that, significant increase in hepatic phosphofructokinase (PFK) and glucose 6 dehydrogenase (G6PDH) was observed. Moreover, the treatment with ACV potentially inhibited key enzymes of lipid metabolism and absorption such as lipase activity in small intestine which led to a notable decrease in serum Total Cholesterol (TC), Low Density Lipoprotein-cholesterol (LDL-c) and triglyceride (TG) rates and an increase in High Density Lipoprotein-cholesterol (HDL-c) levels. The ACV was also observed to protect the liver-kidney functions efficiently, which were evidenced by the significant decrease in the serum aspartate and lactate transaminases (AST and ALT) activities and the level of total and direct bilirubin, creatinine and urea. **Conclusion:** The present findings showed that ACV significant improves glucose and lipid homeostatis in diabetes by delaying carbohydrate and lipid digestion and absorption.

Key words: Cider vinegar, disaccharidases, streptozotocin, glucokinase, lipase

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is one of the most common metabolic diseases and is divided according to the mechanism in which the hyperglycemia is generated into two types, dysfunction in insulin secretion and resistance to its activity. Worldwide, the number of people is exponentially increasing mainly due to aging, urbanization, unhealthy eating habits, increasing prevalence of obesity and lack of physical activity[1]. Hyperglycemia is often associated with serious complications, such as liver and intestine toxicities, renal dysfunction, lipid content rises and alterations, retinopathy and cardiovascular disorders[2-5] and hyperglycemia impaired metabolism of carbohydrate, protein and fat[6]. One of the successful methods for decreasing the onset of diabetes is to control postprandial hyperglycaemia and hyperlipidemia, the inhibition of lipid and carbohydrate hydrolyzing enzymes, such as disaccharidases and lipase in the intestine[7,8]. The line of recent researches has been particularly interested to identifying alternative disaccharidases inhibitors that can reduce the side effects associated with the currently used antidiabetic drugs[9,10]. Apple vinegar as one of the apple products, is a liquid product from fermentation of carbohydrate. Apple cider is widely used in salad and foods. It has been made and used dating from around 300 BC and is an important element in Asian, European, Western and other traditional cuisines of the world[11]. The main component of vinegar is acetic acid being present at concentration of 3-5%[12]. Other many medicinal constituents of vinegar include alcohols, peptides[13,14], polyphenolic compounds (e.g., gallic acid, catechin, caffeic and ferulic acid)[15,16], some vitamins, mineral salt, amino acids and organic acids[17,18]. Some scientific investigations clearly demonstrated several benefits of vinegar such as antimicrobial properties[19,20], prevention of hypertension[21] to stimulation of calcium (Ca) absorption and retention[22], lessening the inflammation[23], lowering serum cholesterol levels[14], treatment of ear infection (otitis external and otitis media)[24,25], treating warts[26], reduction in systolic blood pressure[21] and decreasing the glycemic index of carbohydrate food for people with and without diabetes[27,28].

Accordingly, the present study was undertaken to investigate the antihyperglycemic effects of apple cider vinegar on important enzymes of carbohydrate metabolism in liver and the intestinal disaccharidases and lipase activity profiles of normal and STZ-diabetic rats.

MATERIALS AND METHODS

Animals: The assays of the present study were conducted on adult male Wistar rats, weighing 160-200 g, which were obtained from Siphat, Tunisia. All rats were kept in an environmentally controlled breeding room (temperature: 22±2°C, relative humidity: 55±5%, 12 h dark/light cycle) where they had standard diets (Almes, Mateur, Tunisia) and free access to tap water. The experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals issued by the University of Tunis, Tunisia and approved by the Tunisia Committee of Animal Ethics (approval number: FST/LNFP/Pro 152012).

Induction of experimental diabetes: The overnight-fasted rats were made diabetic with STZ (Sigma chemical company, France) at a dose of 65 mg kg^{-1} intraperitoneally (i.p.). The STZ was freshly dissolved in citrate buffer (0.4 mM, pH 4.5). Control rats received only the buffer. Three days after the injection, diabetes was confirmed in STZ-treated rats with fasting blood glucose above 250 mg dL^{-1} [29].

Experimental procedure: On the day the experiments started and before treatment, the rats were divided into four groups of eight animals each as follows:

Groupe I : Normal untreated rats
Groupe II : Diabetic control rats
Groupe III : Diabetic rats received AVC (0.6% of feed) intragastrically
Groupe IV : Normal rats treated with AVC

Commercial available apple cider vinegar was taken from Vital companies (Boumhel, Tunis, Tunisia). It was diluted with water and it was given to the animals 0.6% of diets[30]. The caloric values of 100 mL apple cider vinegar were 65 kilocalories. It had no protein, fat and fibre. There were small amounts of carbohydrate and sugar, approximately 1%. It was composed of 5% acetic acid and had a pH value of 2.8-3. It contained the following minerals (mg/100 g): Calcium 7, iron 0.2, magnesium 5, phosphorus 8, potassium 73, sodium 5, zinc 0.04, copper 0.008, manganese 0.249 and selenium 0.1 mcg.

One month later, the rats were weighed and sacrificed by decapitation and their trunk blood was collected. The serum was prepared by centrifugation 3,000×g for 10 min at 4°C. After decapitation, the liver was quickly removed, washed in 0.9% NaCl to remove hematoma, blotted individually on ash-free filter paper and weighted. Blood samples were taken for estimation of plasma level of CT, TG, HDL-c, total and direct bilirubin, creatinine, urea, AST and ALT activities. The mucosal small intestine of each rat was excised and the lumen was flushed out several times with 0.9% NaCl. The mucosal

washing and the scraped mucosa were pooled, homogenized and centrifuged (5,000×g for 15 min). The supernatant was frozen and stored for use in subsequent enzymatic assays.

Analytical methods

Blood glucose: At the 3rd and 28th day of the experiment, blood glucose levels of all groups were measured using reagent strips (Accu-Check Active Glucose test strips, Roche, Germany) with a glucometer (Accu-Check Active, Roche, Germany) in samples obtained from the tail vein.

Protein assay: The level of total protein was estimated by the method of Bradford[31] using bovine serum albumin as standard.

Intestinal disaccharidases activities: The activities of intestinal disaccharidases were obtained by measuring the amount of glucose released from various substrates[2]. The intestinal lipase activity was measured using a modified version of the method described by Satouchi *et al.*[32].

Hepatic carbohydrate metabolising enzyme activities: Glucokinase (GK) activity was estimated by the method of Newgard *et al.*[33]. Glucose-6-phosphate dehydrogenase (G6PDH) activity was determined by the method of Bergmeyer[34]. However, phosphofructokinase activity was estimated by the method described by Castano *et al.*[35].

Hepatic glycogen: Glycogen content was determined by the method described by Ong and Khoo[36]. Weighed amounts of liver tissues (0.3-0.5 g) were homogenized in 10 volumes of ice-cold 30% KOH and boiled at 100°C for 30 min. Glycogen was precipitated with ethanol, pelleted, washed and resolubilized in distilled water. Glycogen content was determined by treatment with anthrone reagent and measured at 625 nm.

The activity of aspartate and alanine transaminases (AST and ALT) and the level of TC, TG, HDL-c, total and direct bilirubin, creatinine and urea in serum were measured using commercial kits from Biomagreb, Tunis, Tunisia and Biomerieux, Lyon, France.

The LDL-c level was calculated by using the expression of Friedewald *et al.*[37]:

$$\text{LDL-c (mmol L}^{-1}\text{)} = \text{TC (mmol L}^{-1}\text{)-}$$
$$\text{TG (mmol L}^{-1}\text{)/2.2 -HDL-c; TG<4 mmol L}^{-1}$$

Statistical analysis: Data are presented as Means±SEM. The determinations were performed with 8 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 $p<0.05$ (StatView, SAS Institute, Cary, NC, USA).

RESULTS

Intestinal maltase, sucrase and lactase activities: Results indicated that compared to the control rats, there was a significant increase ($p<0.05$) in the activities of three disaccharidases: (A) Maltase, (B) Sucrase and (C) Lactase in the intestine of STZ-diabetic rats. However, after ACV administration, a considerable reduction ($p<0.05$) of these intestinal disaccharidases activities was observed in normal and STZ-diabetic animals (Fig. 1).

Plasma glucose level, hepatic glycogen content and hepatic glucokinase (GK), glucose 6 phosphate dehydrogenase (G6PDH) and phosphofructokinase (PFK) activities.

Table 1 shows the effects of administering ACV to normal and surviving STZ-diabetic rats on plasma glucose concentration, glycogen content, GK, G6PDH and PFK activities in the livers of the control and experimental groups of the rats. In fact, a considerable decrease ($p<0.05$) in both G6PDH and PFK activities as well as in the glycogen content and a concomitant increase in the activity of hepatic GK were noted in the livers of the untreated diabetic group of rats. The ACV administration to normal and diabetic rats resulted in a marked elevation of G6PDH and PFK activities and restored the liver glycogen content to near normal levels. In addition, the administration of ACV to diabetic rats was observed to reduce the plasma glucose concentration and the hepatic GK activity as compared to the untreated STZ-diabetic rats (Table 1).

Intestinal lipase activity and plasma lipid concentration: Figure 2 indicate that, the intestinal lipase activity (A) underwent a potent increase ($p<0.05$) in diabetic rats when compared to the control rats. The TC (B), triglycerides (C) and LDL-c (D) concentrations in plasma witnessed also a significant raise ($p<0.05$) in the STZ-diabetic rats. However, the admnistration of ACV to the normal and surviving diabetic rats was observed to have reverted the activity of lipase in intestine (A). This supplement also led to a considerable decrease in TC (B), triglycerides (C) and LDL-c (D) concentrations in plasma. It was also observed that streptozotocin induced a decrease ($p<0.05$) in the HDL-c (E) concentration and that ACV prevented this decrease (Fig. 2).

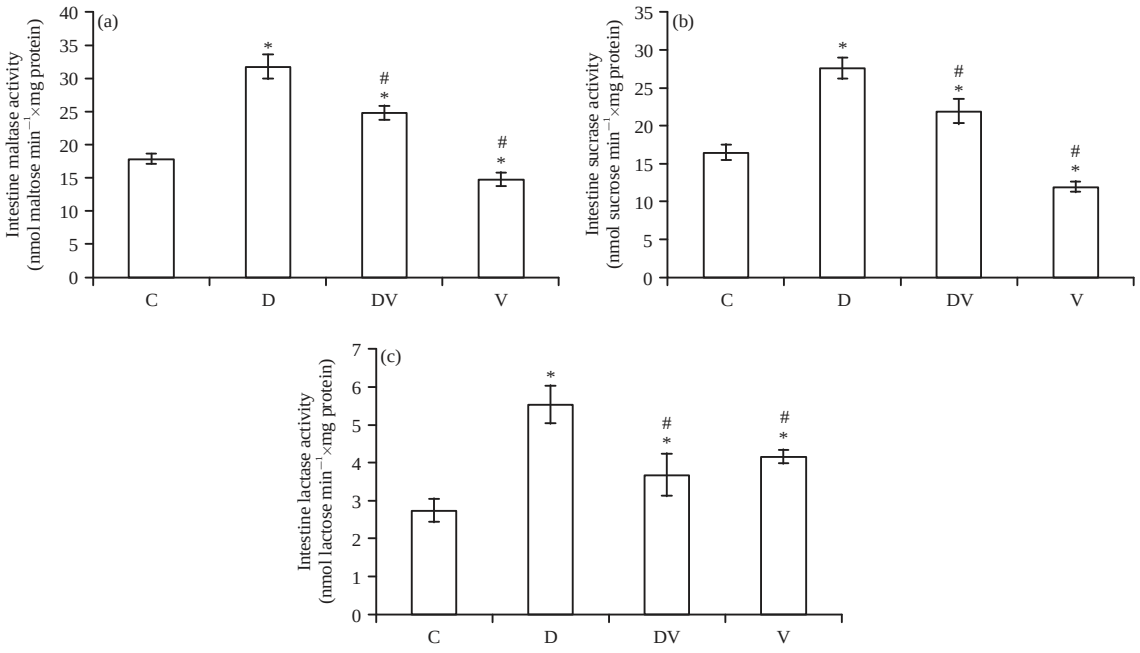

Fig. 1(a-c): Effect of ACV on (a) Maltase, (b) Sucrase and (c) Lactase activities in the small intestine of normal and STZ-diabetic rats. Values are given as Mean±SD for group of 8 animals each. Values are statistically significant at *p<0.05 compared to control rats and #p<0.05 compared to diabetic rats

Table 1: Blood glucose and glycogen hepatic levels and GK, G6PDH and PFK activities in normal and STZ-diabetic rats treated with ACV

	C	D	DV	V
Blood glucose level (g L^{-1})	1.32±0.02	3.28±0.07*	2.43±0.10*#	1.18±0.02*#
Glycogen hepatic level (mg g^{-1} tissue)	2.89±0.05	1.04±0.04*	1.99±0.03*#	3.57±0.09*#
Glucokinase activity (nkat mg^{-1} protein per gram tissue)	51.21±2.06	122.74±3.78*	112.75±2.11*#	49.58±11.6#
G6PDH activity (nkat mg^{-1} protein per gram tissue)	37.80±0.42	21.35±0.47*	39.37±0.51#	41.54±0.7*#
Phosphofructosamine activity (nkat mg^{-1} protein per gram tissue)	750.25±6.32	602.62±5.23*	655.10±3.52*#	772.78±3.73*#

Values are given as Mean±SD for group of 8 animals each. Values are statistically significant at *p<0.05 compared to control rats and #p<0.05 compared to diabetic rats

Hepato and kidney toxicity indices in plasma (AST, ALT, total and direct bilirubin, urea and creatinine): Figure 3 shows that streptozotocin provoked hepato and renal toxicity. It was noted also that ACV prevented the liver from hepatotoxicity and reduced the levels of AST (A) and ALT (B) as well as total (C) and direct bilirubin (D) as compared with those of unsupplemented STZ-treated rats. In addition, ACV reduced the state of nephropathology by normalizing the renal toxicity indices (urea (E) and creatinine (F)) (Fig. 3).

DISCUSSION

The inhibition of intestine disaccharidases by food containing polysaccharides has been reported to retard the digestive process throught their inhibition of intestinal digestive enzymes and this was observed to bring about better control of hyperglycemia[38,39]. The present findings showed that the administration of ACV to normal and STZ-diabetic rats significantly decreased maltase, sucrase and lactase activities present as key intestinal enzymes and involved in the conversion of oligosaccharides into monosaccharides. This inhibitory effects exhibited by intestinal disaccharidases seen to have limited the process of carbohydrate hydrolysis and absorption in the intestine. In fact, the results of the present study are in accordance with the data reported by Ogawa *et al.*[40] who demonstrated that the hypoglycemic impacts of acetic acid, which is the active ingredient of vinegar have been shown to be mediated in the supression of disaccharidases activity in human intestinal cells. The antihyperglycemic properties of apple vinegar were firstly reported by Ebihara and Nakajima[41]. In animals, at concentrations found in traditional diets, it has varying effects such as enhancement of glycogen repletion[42]. Ostman *et al.*[43] demonstrated that white vinegar reduced both

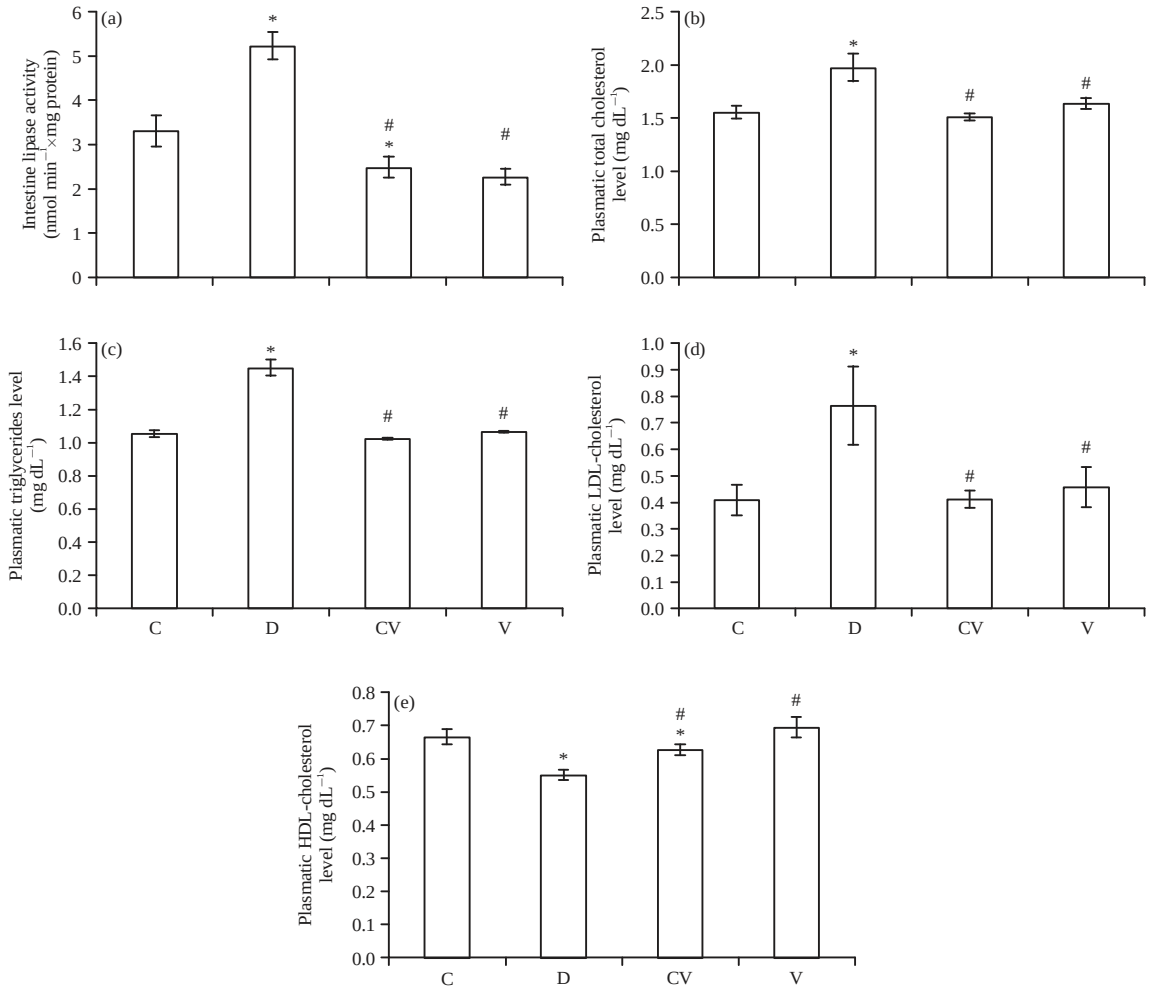

Fig. 2(a-e): (a) Intestinal lipase activity, (b) Serum lipid profile TC, (c) TG, (d) LDL-c and (e) HDL-c in control and STZ-diabetic rats treated with ACV, values are given as Mean±SD for group of 8 animals each. Values are statistically significant at *p<0.05 compared to control rats and #p<0.05 compared to diabetic rats

postprandial blood and insulin levels. In insulin resistant subjects, ACV indicated to improve postprandial insulin sensitivity[28]. Acetic acid may control these factors via different manners like slowing-down of gatric emptying[44], inhibition of disaccharidases activity in the small intestine, blocking the complete digestion of starch molecules[40] and also promotion of glucose uptake by muscle performance[42]. Liver plays an important role in defense the postprandial hyperglycemia and synthesis of glucose metabolism. In glucose utilisation, it converts the glucose-6-phosphate that increases the production of fats to carbohydrates that turn to deposition into the liver and the kidney. It also altered the level of hexokinase, which decreases the conversion and utilization of glucose. The STZ-induced diabetic groups treated with ACV decreased the level of glucokinase and increased the level of G6PDH and phosphofructokinase and brought the level near the normal group levels. The results showed that decreased glucose levels in normal and diabetic animals may be correlated with the inhibition of glycogenolysis as suggested by increased liver glycogen indicating the insulin secretagogue activity. Glycogen level in various tissues especially in liver and skeletal muscle indicates direct reflection of insulin activity since it causes glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Glycogen levels in tissues (muscle and liver) decrease as the influx of glucose in the liver is inhibited in the absence of insulin and recovers on insulin treatment[45]. Together, the decrease in the activities of disaccharidases in the intestine, which are essential enzymes for terminal absorption of carbohydrate digestion through the conversion of disaccharides to readily soluble monosaccharides as well as the modulation of some

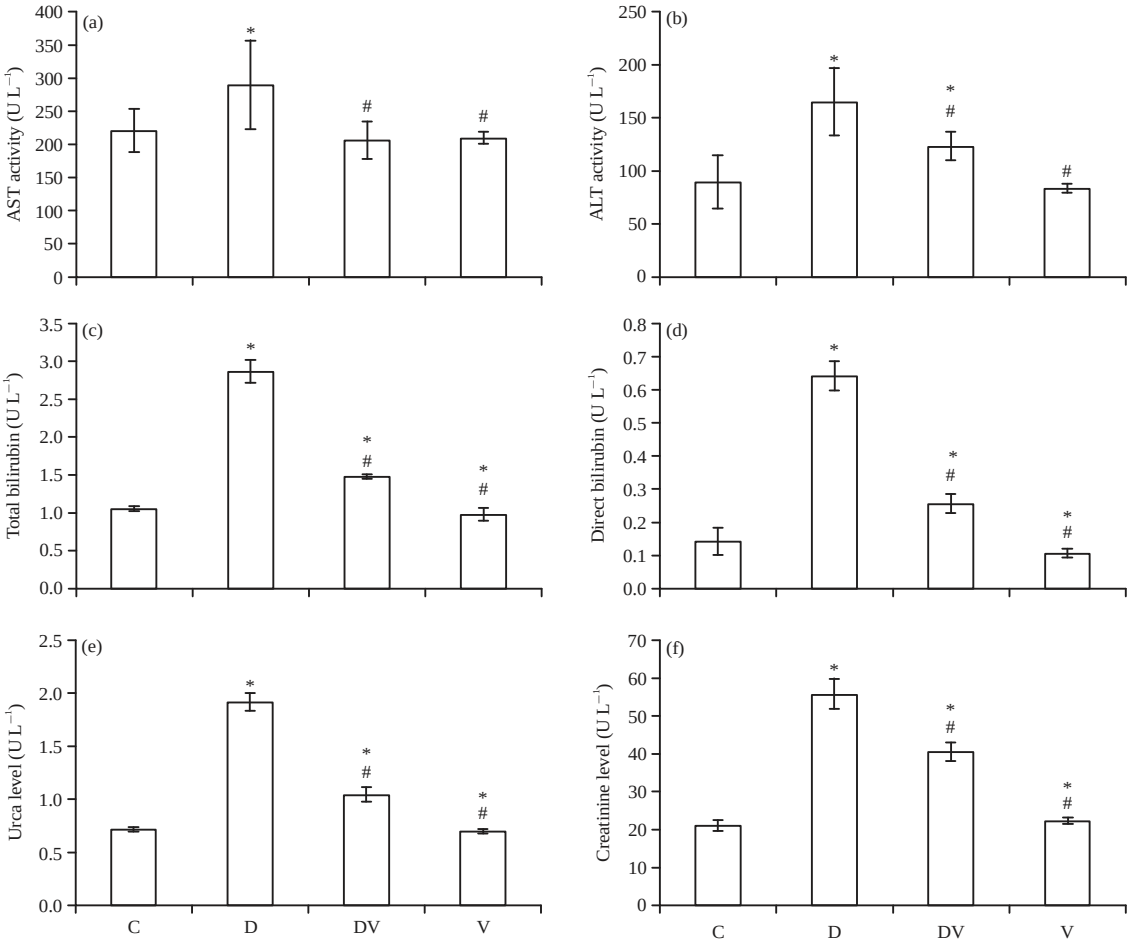

Fig. 3(a-f): Values of indices of hepatic (a) AST, (b) ALT, (c) Total, (d) Direct bilirubin, renal (e) Urea and (f) Creatinine toxicity in control and STZ-diabetic rats treated with AVC, values are given as Mean±SD for group of 8 animals each. Values are statistically significant at *p<0.05 compared to control rats and #p<0.05 compared to diabetic rats

carbohydrate activities such as GK, G6PD and PFK in the livers led to considerable decrease in blood glucose level in rats treated by ACV.

Despite these hypoglycemic effects, ACV has reno and hepatoprotective effects as well. The hyperglycemia also induces the elevation of the plasma levels of urea and creatinine which are considered as signi cant markers of renal dysfunction[46]. After oral admnistration of ACV a decreased in creatinine and urea levels p<0.05 in STZ-diabetic and normal rats were observed. This results may be due to the high levels of phenolic compounds in ACV specifically catechin and pyrogallol, which prevent kidney from the destruction induced by diabetic diseases. Pitchai and Manikkam[47] reported that the administration of catechin lowered urea and creatinine in diabetic rats. The effect of ACV on liver function is illustrated in Fig. 3. The indices of hepatic toxicity (ALT, AST, total bilirubin and direct bilirubin) increased in diabetic group compared to the control group. However, after ACV administration, a considerable reduction in the levels of the liver transaminases (ALT and AST) as well as total and direct bilirubin was observed, suggesting that ACV may play an important role in preventing the liver from hepatotoxicity.

Treatment with ACV improves lipid profile since it significantly decreases serum TC, TG and LDL-c levels and increases HDL-c which became so pronounced after 4 week of treatment. These findings are in accordance with the result of Shishehbor *et al.*[30] who reported that AVC administration to normal and diabetic rats improved the serum lipid profile. This is consistent with the findings of Bender *et al.*[48] who reported that oral administration of ACV to normal mice induced a significant reduction in plasma TG levels. Futhermore, Fushismi *et al.*[14] have reported that acetic acid lowered serum TG in rats which were fed a cholesterol-rich diet. However, higher plasma TG concentrations in diabetic

mice treated which acetic acid were observed by Sakakibara *et al.*[12] while TG levels in the liver was significantly decreased. The hypotriglyceridemic effect of AVC might be due to the reduction of hepatic TG storage[48]. This could be further supported 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 beta-oxidation.

The observed reduction in plama triglyceride seen in diabetic rats was paralleled by increased plasma HDL-c concentration. Futhermore, ACV lowered serum LDL-c and increased serum HDL-c in normal rats. These results were also induced by white vinegar in normal rats in the experiment of Shishehbor *et al.*[49]. The possible mechanism attribued to these findings could be related to the lowering effect of vinegar/or acetic acid on the glycemic index[43,27]. It has been found that the lower glycemic index diets are able to increase HDL-c and reduce LDL-c levels[50,51]. Futhermore, the low glycemic diet has been shown to decrease serum LDL-c concentrations in diabetic patients[52]. Ford and Liu[53] also reported an inverse relation between dietary glycemic index and plasma HDL-c concentrations in adults. Apple polyphenols have been shown to decrease the serum LDL-c levels in healthy human[54] and increase the serum HDL-c in rats[55] and in hamsters[56]. These changes in HDL-c and LDL-c concentrations could be possibly contributed to the suppression of intestinal lipoprotein secretion by apple polyphenols[57]. These results suggest that ACV may improve lipoprotein pattern not only by lowering the glycemic index but also by its polyphenolic compounds.

In addition, intestinal lipase inhibitors are considered to be a valuable therapeutic reagent for treating diet-induced obesity in humans. Hamden *et al.*[58] and Dahlqvist[2] demontrated that diabetes increased lipase activity in the intestine and that the increased lipase absorption from the intestine consequently amplified the hypercholesterolemia and hyperlipidemia effects. The inhibitory action of lipase in the intestine decreased the hydrolysis of dietary triglycerides to monoglycerides and free fatty acids as it lowered the lipid level of the blood[2,58]. The administration of ACV to surviving diabetic rats also exerted *in vivo* inhibitory effects of key enzymes of lipid digestion and absorption such as lipase in the small intestine and hydrolyses non-absorbable triglycerides into simple glycerol and fatty acids absorbable by the small intestine. The results of the present study demonstrated that the administration of ACV to surviving diabetic rats, clearly reverted the activity of intestinal lipase nearly back to that of the non diabetic rats. The inhibitory action of lipase in the intestine decreased the hydrolysis of dietary TG into monoglycerides and free fatty acids as it lowered of HDL-c levels in serum. The present findings showed that vinegar inhibited the lipase activity and resulted in the suppression of triglycerides digestion and thus increasing fecal elimination of fat and therefore overall effects resulted in a weight loss and improved the lipid profile.

CONCLUSION

Therefore, the findings of the present study provide sheer evidence that apple cider vinegar has a promisingly efficient potency as a therapeutic agent and can, therefore, be considered as potential strong candidate for future biotechnological applications interested in hypoglycemic and hypolipidemic drug development.

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