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Effects of Apple Cider Vinegars Produced with Different Techniques on Blood Lipids in High-Cholesterol-Fed Rats

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ABSTRACT: Red delicious apples were used to produce natural apple cider with and without inclusion of maceration. Traditional surface and industrial submersion methods were then applied to make vinegar from apple ciders. Apple cider vinegar samples produced with inclusion of maceration in the surface method had the highest total phenolic content, chlorogenic acid, ORAC, and TEAC levels. Cholesterol and apple vinegar samples were administered using oral gavage to all groups of rats except the control group. Apple cider vinegars, regardless of the production method, decreased triglyceride and VLDL levels in all groups when compared to animals on high-cholesterol diets without vinegar supplementation. Apple cider vinegars increased total cholesterol and HDL and LDL cholesterol levels and decreased liver function tests when compared to animals on a high-cholesterol diet without vinegar supplementation. A high-cholesterol diet resulted in hepatic steatosis. VSBM and VSB groups significantly decreased steatosis.

KEYWORDS: apple cider vinegar, antioxidant activity, high-cholesterol-fed rat, blood lipids, HDL, body weight

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of mortality worldwide. High blood cholesterol levels, high blood pressure, atherosclerosis, unhealthy diet, obesity, smoking, and diabetes may cause cardiovascular disease.[1,2]

Cholesterol and blood triglyceride concentrations are regulated by genetic factors and diet.[3] Foods containing high amounts of saturated fat may adversely affect the lipid profile of blood.[4] Consumption of fruits and vegetables in the diet should be increased because they have high content of polyphenolic compound, which are known as antioxidants.[5] Polyphenolics have been claimed to play an important role in affecting human health by preventing several diseases including cancer, hypertension, heart attack, and diabetes.[6]

Consumption of foods derived from fruits and vegetables is also essential; fruit juices, wines, and vinegars also contain significant amounts of polyphenolic compounds. Vinegar is obtained mainly from different varieties of wine by two fermentation steps, ethanol and acetic acid fermentations. There are mainly two vinegar production methods. Followed by wine production; one is a surface method in which the culture of acetic acid bacteria is inoculated on the surface of a barrel, also known as the traditional method. The second method is a submersion technique involving a submerged culture by which the oxygenation has been greatly improved (industrial method).[7]

Vinegar is obtained from grape, apple, and other fruit juices with sugar.[8] Apple and apple products (juice, cider, vinegar) are commonly consumed worldwide. Apple polyphenols contain mainly polyphenolic acid derivatives and other flavonoids. Vinegars usually have the higher content of polyphenolics, which are gallic acid, epicatechin, catechin, tyrosol, benzoic acid, syringic acid, vanillin, caftaric acid, coutaric acid, chlorogenic acid, caffeic acid, coumaric acid, and ferulic acid.[9,10] Foods rich in chlorogenic acid inhibit DNA damage in vitro[11] and show a protective effect against cardiovascular diseases with inhibition of LDL oxidation.[12] Positive health effects such as increasing intestinal calcium absorption,[13] reduction of blood pressure,[14] positive effects on blood glucose response, and serum insulin[15] of various vinegars were reported. In addition, a potential antitumor effect of apple cider vinegars has been noted.[16]

The purpose of the study was to determine the effects of production methods (submerged and surface methods) on chemical composition, total antioxidant activity and phenolic substances of apple cider vinegars. The other important aim of the study was to find the effects of apple cider vinegars on blood lipids, liver functions, glucose levels, and body weight of high-cholesterol-fed Wistar rats. To our knowledge, this is the first report regarding the effects of apple cider vinegar on blood lipids, liver functions, and body weight.

MATERIALS AND METHODS

Chemicals. Folin−Ciocalteu reagent was supplied by Merck (Darmstadt, Germany). 2,2′-Azinobis(3-ethylbenzthiazolin-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), fluorescein disodium salt, and gallic acid were purchased from Acros (Morris Plains, NJ). Standards (catechin, epicatechin, chlorogenic acid, caffeic acid, *p*-coumaric acid) for qualification of phenolics using HPLC and cholesterol were purchased from Sigma (Milan, Italy).

Apple Cider Vinegar Production. 'Red Delicious' apples were used to make natural apple cider. To determine the effects of maceration and production methods on properties of vinegars, the following

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Table 1. Statistical Evalution of the Calibration Data of Gallic Acid, Catechin, Epicatechin, Chlorogenic Acid, Caffeic Acid and *p*-Coumaric Acid by RP-HPLC

phenolic	linearity range (μg/mL)	slope	intercept	correl coeff	LOD (μg/mL)	LOQ (μg/mL)
gallic acid	0.34−0.085	61910	6.2	0.9996	0.02	0.05
catechin	0.93−0.23	18292	15.3	0.9991	0.07	0.21
chlorogenic acid	0.34−0.08	57270	18.2	0.9999	0.004	0.012
caffeic acid	0.21−0.054	119119	21.4	0.9999	0.001	0.004
epicatechin	0.875−0.21	20933	22.5	0.9998	0.02	0.06
p-coumaric acid	0.07−0.01	543764	29.3	0.9998	0.002	0.006

Table 2. Experimental Design

group	treatment	code
1	rat chow (control) (n = 9)	CNT
2	high-cholesterol diet (cholesterol control) (n = 9)	CHOLCNT
3	high-cholesterol diet + apple cider vinegar produced using surface method with maceration (n = 9)	VSTM
4	high-cholesterol diet + apple cider vinegar produced using surface method without maceration (n = 9)	VST
5	high-cholesterol diet + apple cider vinegar produced using submersion method with maceration (n = 9)	VSBM
6	high-cholesterol diet + apple cider vinegar produced using submersion method without maceration (n = 9)	VSB

vinegars were produced: apple cider vinegar produced using a surface method including maceration (VSTM), apple cider vinegar produced using a submersion method including maceration (VSBM), apple cider vinegar produced using a surface method (VST), and apple cider vinegar produced using a submersion method (VSB). Addition of 10% pomace was used in the maceration step to increase the polyphenolic contents. Apple cider vinegars were produced in the facilities of Suleyman Demirel University, Department of Food Engineering, in cooperation with the Carl Kuhne Vinegar Plant. The production was carried out according to the method of Budak and Guzel-Seydim.[17]

Compositional Analysis. Total titratable acidity and total solid contents of vinegar samples were measured according to AOAC methods. Vinegar samples were titrated with standardized 1 N NaOH to the end point of pH 8.1. Total titratable acidity was expressed as acetic acid equivalent. pH was measured with an Inolab pH-meter (WTW Measurement System, FL, ABD). Density, total solids, water-soluble solids (°Brix), and total ash were also determined in vinegar samples according to AOAC methods.[18]

ABTS Assay. $ABTS^+$ radical cation was prepared by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate.[19] $ABTS^+$ inhibition against Trolox was spectrophotometrically measured.[20] The absorbance was measured at 734 nm in a spectrophotometer (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). TEAC values of samples were calculated from the Trolox standard curve and expressed as Trolox equivalents (in mmol L^{-1} of sample).

Oxygen Radical Absorbance Capacity (ORAC) Assay. Vinegar samples were analyzed using the ORAC assay by Wu et al.[21] The samples were appropriately diluted with phosphate buffer (pH 7.4) for ORAC analysis. An aliquot (25 μL) of the diluted sample, blank (phosphate buffer), or Trolox calibration solution was added to a black, clear-bottom triplicate well in a 96-well bottom reading microplate. After the addition of 150 μM fluorescein stock solution (0.004 μM) to each well, the microplate was incubated at 37 °C for 30 min. Then, 25 μL of AAPH solution (153 mM) was added to start the reaction. The microplate reader was programmed to record the fluorescence reading with an excitation−emission wavelength of 485−520 nm using Gen 5 software. Antioxidant activity was kinetically measured with a Biotek Synergy HT Multi-Detection Microplate Reader (Winooski, VT).

Total Phenolic Content. Total phenolic contents of vinegar samples were determined according to the Folin−Ciocalteu method using gallic acid as a standard.[22] After the addition of Folin−Ciocalteu reagent to the sample solution, it was allowed to react for 6 min. Reaction was stopped by using 1.50 mL of 20% sodium carbonate. The color was developed in 120 min in a dark place, and the absorbance was determined at 760 nm using a spectrophotometer (Shimadzu Scientific Instruments, Inc.). The measurement was calculated using a standard curve of gallic acid and expressed as milligrams of gallic acid equivalents (GAE) per liter.

Quantification of Phenolics by High-Performance Liquid Chromatography. A high-performance liquid chromatography (Shimadzu, Kyoto, Japan) was used for the identification and quantification of compounds in vinegar samples. This system consisted of a model LC-10ADvp pump, an SIL-10AD vp autosampler, a DAD detector (λ_{max} = 278), an SCL-10Avp system controller, a DGU-14A degasser, a CTO-10Avp column oven, and an Agilent Eclipse XDB-C18 (250 × 4.60 mm) 5 μm column. The chromatographic conditions for the samples were as follows: flow rate, 0.8 mL min^{-1}; injection volume, 20 μL; column temperature, 30 °C. Methanol and acetic acid (3%) solvents were used as mobile phase. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. Separate standard calibration graphs were constructed for each component by plotting the peak area of the phenolic compounds against the phenolic compound concentration (Table 1). These results showed highly reproducible calibration curves with correlation coefficients of >0.999. The low values of SE of slope, intercept, and >0.999 correlation coefficient for all compounds established the precision of the proposed methods. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from equations using the standard deviation (s) of response and the slope (m) of the corresponding calibration curve.

Design of Rat Experiments. The Animal Care Ethical Committee of Suleyman Demirel University approved this study. Research was carried out at the Animal Production and Research Center in Suleyman Demirel University, Isparta, Turkey. Fifty-four male Wistar albino rats weighing between 200 and 250 g were included in this study. The rats were kept in a room with a 12 h light−dark cycle. The temperature was controlled at 24 °C, and the rats were given ad libitum access to food and water. Rats were divided into six groups of nine. One milliliter of 2.5% cholesterol and 1 mL of diluted apple cider vinegar (157 μL of apple cider vinegar in 843 μL of distilled water; this ratio was used to obtain 1% total acidity to avoid excess acetic acid) had been given daily by oral

Table 3. Chemical Properties of Vinegars Produced with Different Techniques[a]

vinegar	total acidity (g/L)	pH	density	total solids (%)	°Brix	total ash (g/L)
VSTM	57.17 ± 1.66 b	2.87 ± 0.05 b	1.0201 a	19.68 ± 1.62 a	5.5 ± 0.35 a	2.02 ± 0.12 c
VSBM	73.86 ± 2.88 a	3.16 ± 0.03 a	1.0225 a	18.02 ± 0.70 a	6.00 ± 0.0 a	4.74 ± 0.31 a
VST	55.16 ± 1.13 b	2.83 ± 0.02 b	1.0208 a	12.01 ± 0.74 b	4.83 ± 0.16 a	1.75 ± 0.00 c
VSB	73.83 ± 1.34 a	3.21 ± 0.08 a	1.0239 a	12.96 ± 1.93 b	5.00 ± 0.5 a	4.05 ± 0.03 b

[a] Results are expressed as the mean ± SEM. Values with the same lower case letters in the same column do not differ significantly ($P > 0.05$). VSTM, apple cider vinegar produced with using the surface method including maceration; VSBM, apple cider vinegar produced using submersion method including maceration; VST, apple cider vinegar produced with using the surface method;VSB, apple cider vinegar produced using the submersion method.

gavage[23] for 7 weeks; vinegar was given at 9 a.m., and cholesterol was given at 5 p.m. The control diet (CNT) group received the same volume of physiological saline (1 mL) at the same time at the experiment groups. The animal test design is presented in Table 2. Body weights of all animals were recorded weekly. Rats were anesthetized with intraperitoneal 2% xylazine (10 mg/kg) and 10% ketamine (80 mg/kg) at the end of the experiment. Blood samples were taken from the inferior vena cava, and rats were sacrificed with exsanguinations. Liver and stomach specimens were taken and fixed in 10% formaldehyde and embedded in paraffin for histopathological examination.

Blood samples were centrifuged for 5 min at 2500*g*, and serum supernatants were separated and studied immediately for biochemical analysis.

Biochemical Analyses. Concentrations of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), glucose (Glu), total bilirubin (TBil), indirect bilirubin (IBil), direct bilirubin (DBil), aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were measured spectrophotometrically using an Olympus AU 2700 autoanalyzer (Tokyo, Japan) in serum samples with commercially available specific kits. TC, TG, HDL, LDL, VLDL, Glu, TBil, DBil, and IBil were expressed as milligrams per deciliter, and AST, ALT, and ALP activities were expressed as units per liter.

Histopathological Examination. Specimens for histopathological examination of liver and stomach were fixed in 10% formaldehyde, embedded in paraffin, cut in 5 μm thicknesses, and stained with hematoxylin−eosin (H&E). These sections were evaluated using an Olympus BX51 microscope for histopathological changes. The histological examination included semiquantitative analysis of steatosis (fat accumulation) in the liver. All sections were evaluated by the same pathologist, who had no knowledge of the experimental design. The steatosis was graded on a scale from 0 to 4+, as follows: 0 = absence of steatosis, 1+ = focal (<50% of lobule central veins) steatosis, 2+ = steatosis (>50% of lobule central veins), 3+ = comprehensive steatosis, 4+ = comprehensive and intense steatosis.[24]

Statistics Analysis. All data were reported as the mean ± SEM and analyzed by using SPSS for Windows (version 17.0, SPSS Inc.). The statistical analysis among the groups was done by Kruskall−Wallis test, followed by Mann−Whitney *U* test as a post hoc for the pairwise comparisons. All tests were two-sided, and the differences among groups were considered to be statistically significant when probability values were <0.05.

RESULTS

Compositions and Antioxidant Activities of Apple Cider Vinegars. Total titratable acidity, pH, density, total solids, water-soluble solids, and total ash in vinegar samples are given in Table 3. Total acidity and pH values of samples had similar tendencies; different vinegar production methods resulted in significant differences ($P < 0.05$). Whereas the pH values of vinegars produced using the surface method were between 2.87 and 2.83, the pH values of vinegar samples produced using the submersion method were between 3.16 and 3.21 ($P < 0·05$). VSTM had the highest total solids among the samples ($P < 0.05$) (Table 3). Water-soluble solids of vinegar samples were similar ($P > 0.05$). Total ash contents of the samples ranged between 1.75 and 4.74 g/L.

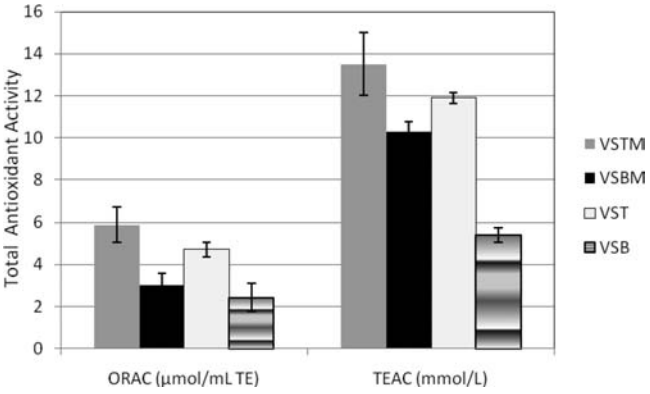

Figure 1. Total antioxidant activity of TEAC and ORAC assays of vinegar samples.

Total phenolic content, TEAC, and ORAC results express the total antioxidant activity in apple cider vinegars produced with different techniques. The total phenolic contents and antioxidant activity of apple cider vinegars produced with the surface method were higher than the total phenolic content and antioxidant activity of apple cider vinegars produced with the submersion method ($P < 0.05$). In our study, ORAC and TEAC values of VSTM were 5.89 μmol/mL and 13.5 mmol/L, respectively. ORAC values of VSBM, VST, and VSB samples were 3.00, 4.71, and 3.99 μmol/mL, respectively. TEAC values of VSBM, VST, and VSB samples were 10.27, 11.90, and 5.4 mmol/L, respectively (Figure 1). Total phenolic contents of VSTM, VSBM, VST, and VSB samples were 908.595L, 568.60, 757.65, and 416.95 mg/L, respectively. VSTM had the highest total phenolic content among vinegar samples ($P < 0.05$).

Gallic acid, catechin, epicatechin, caffeic acid, chlorogenic acid, and *p*-coumaric acid were detected in apple cider vinegars (Table 4). Apple cider vinegars had low contents of gallic acid, catechin, epicatechin, caffeic acid, and *p*-coumaric acid. Chlorogenic acid was the dominant phenolic substance in apple cider vinegar; especially, VSTM had the highest content of chlorogenic acid. *p*-Coumaric acid contents of vinegars ranged between 0.05 and 0.07 mg/L.

Biochemistry Results of Rats. The serum levels of TG, TC, HDL, LDL, and VLDL of all groups are shown in Table 5.

Table 4. Total Phenolic Content and Phenolic Compounds of Apple Vinegars Produced with Different Techniques[a]

vinegar	total phenolic content (mg/L)	gallic acid (mg/L)	catechin (mg/L)	epicatechin (mg/L)	caffeic acid (mg/L)	chlorogenic acid (mg/L)	*p*-coumaric acid (mg/L)
VSTM	908.595 a		0.86 ± 0.03 b	1.40 ± 0.32 b	1.00 ± 0.15 a	18.67 ± 2.77 a	0.05 ± 0.01 a
VSBM	568.60 c	0.05 ± 0.05 b	0.80 ± 0.15 b		0.60 ± 0.10 b	2.40 ± 0.72 b	0.07 ± 0.01 a
VST	757.65 b			0.70 ± 0.10 c		14.83 ± 0.78 a	0.06 ± 0.00 a
VSB	416.95 c	1.00 ± 0.31 a	1.00 ± 0.35 a	2.00 ± 0.75 a	0.56 ± 0.06 b	13.25 ± 1.75 a	0.07 ± 0.00 a

[a] Results are expressed as the mean ± SEM. Values with the same lower case letters in the same column do not differ significantly ($P > 0.05$). VSTM, apple cider vinegar produced using the surface method including maceration; VSBM, apple cider vinegar produced using the submersion method including maceration; VST, apple cider vinegar produced using the surface method;VSB, apple cider vinegar produced using the submergsion method.

Table 5. Lipid Profiles Results of All Treatment Groups[a]

group	TG	TC	HDL	LDL	VLDL
CNT	25.43 ± 2.52	47.14 ± 2.26	36.43 ± 1.54	5.29 ± 0.87	5.00 ± 0.53
CHOLCNT	41.00 ± 4.14 *	67.38 ± 2.07 *	41.63 ± 1.08 *	16.25 ± 1.25 *	8.25 ± 0.92 *
VSTM	35.14 ± 2.85 *	92.43 ± 5.85 **	56.28 ± 2.62 **	26.29 ± 5.15 **	7.00 ± 0.58 *
VST	26.43 ± 3.32 **	91.29 ± 6.49 **	46.29 ± 2.94 **	40.57 ± 6.80 **	5.29 ± 0.64 **
VSBM	31.43 ± 3.01	92.29 ± 5.79 **	47.14 ± 1.72 **	39.14 ± 5.18 **	6.29 ± 0.52
VSB	36.43 ± 4.82 *	111.00 ± 6.28 **	47.00 ± 2.56 **	56.00 ± 6.25 **	7.14 ± 0.91 *

[a] Results are expressed as the mean ± SEM. *, $P < 0.05$ when compared to CNT; **, $P < 0.05$ when compared to CHOLCNT.

Table 6. Liver Function Test Results of All Treatment Groups[a]

group	AST (U/L)	ALT (U/L)	ALP (U/L)	TBil (mg/dL)	DBil (mg/dL)	IBil (mg/dL)	Glu (mg/dL)
CNT	158.11 ± 7.74	40.44 ± 2.78	133.11 ± 15.47	0.07 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	73.22 ± 3.78
CHOLCNT	188.4 ± 21.59 *	53.00 ± 3.87 *	196.89 ± 22.01 *	0.10 ± 0.02	0.05 ± 0.00	0.06 ± 0.02	87.10 ± 4.69 *
VSTM	140.11 ± 12.40 **	38.33 ± 3.07 **	151.00 ± 11.94	0.09 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	123.44 ± 10.60 *,***
VST	150.11 ± 33.07 **	43.87 ± 2.96 **	144.87 ± 16.33 **	0.13 ± 0.01	0.03 ± 0.00	0.09 ± 0.01	108.43 ± 10.87 *
VSBM	107.11 ± 7.04 **	40.44 ± 1.78 **	169.56 ± 20.40	0.11 ± 0.01	0.03 ± 0.00	0.07 ± 0.00	95.22 ± 6.36 *
VSB	98.11 ± 17.16 **	38.89 ± 0.99 **	124.55 ± 8.95 *	0.10 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	87.88 ± 3.61 *

[a] Results are expressed as the mean ± SEM. *, $P < 0.05$ when compared to CNT; **, $P < 0.05$ when compared to CHOLCNT.

Serum levels of TG, TC, HDL, LDL, and VLDL were significantly increased in rats fed the high-cholesterol diet (CHOLCNT) when compared to control (CNT) ($P < 0.05$). Serum levels of TG and VLDL were decreased in all groups given apple cider vinegar (VSTM, VST, VSBM, and VSB) when compared to high-cholesterol-fed animals (CHOLCNT), although it is not statistically significant. TC, HDL, and LDL levels were increased in all groups given apple cider vinegar (VSTM, VST, VSBM, VSB) when compared to the high-cholesterol-fed animals (CHOLCNT). The increase of TC level was significant in all apple cider vinegar groups ($P < 0.05$). However, the increase of HDL level was significant in only the VSTM group ($P < 0.05$), and the increase in LDL level was significant in the VST, VSBM, and VSB groups ($P < 0.05$).

The serum levels of AST, ALT, ALP, TBil, DBil, IBil, and Glu are summarized in Table 6. Serum bilirubin levels (TBil, DBil, and IBil) were not altered in all experimental groups ($P > 0.05$). Serum AST, ALT, ALP, and glucose levels were significantly increased in high-cholesterol-fed animals (CHOLCNT) when compared to controls ($P < 0.05$). Serum levels of AST, ALT, and ALP were decreased significantly in all groups receiving apple cider vinegar (VSTM, VST, VSBM, and VSB) when compared to high-cholesterol-fed animals (CHOLCNT) ($P < 0.05$). Blood glucose levels were significantly higher than the control group in rats given apple cider vinegar along with a high-cholesterol diet.

Table 7. Rat Body Weights of All Treatment Groups[a]

group	body weight (g) 1 week	body weight (g) 7 weeks	*P*
CNT	258.33 ± 4.99	282.66 ± 7.34	<0.05
CHOLCNT	275.50 ± 6.11	294.10 ± 6.29	<0.05
VSTM	255.00 ± 4.62	279.77 ± 8.77	<0.05
VST	271.00 ± 9.00	288.37 ± 7.53	>0.05
VSBM	267.77 ± 5.71	288.77 ± 7.53	<0.05
VSB	269.33 ± 6.77	282.00 ± 10.55	>0.05

[a] Results are expressed as the mean ± SEM.

Body weight differences before and after the experiment of all groups are shown in Table 7. All rats included in this study had gained weight at the end of the experiment. The increase in body weight was not significant in groups VST and VSB, whereas the weight gained in CNT, CHOLCNT, VSTM, and VSBM groups was significant.

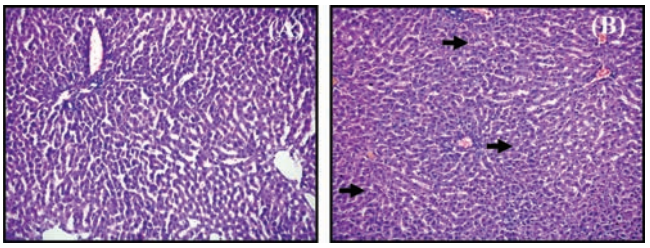

Figure 2. Hematoxylin−eosin-stained section of rat liver in the CNT group (A) and in the CHOLCNT group (B). Arrows indicate lipid pockets. Magnification 200×.

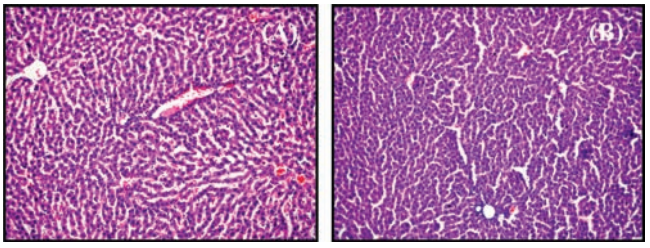

Figure 3. Section of rat liver in the CHOLCNT group (A) and in the VSBM group (B). Magnification 200×.

Histopathology Results of Rats. A significant steatosis was seen in rats fed the high-cholesterol diet (CHOLCNT) when compared to the control group (CNT) ($P < 0.05$) (Figure 2).

Apple cider vinegars produced using the submersion method (with or without maceration) showed significantly decreased steatosis in groups VSBM and VSB groups when compared to the CHOLCNT group ($P < 0.05$; Figure 3).

Stomach specimens of all rats from all groups were similar and normal (Figure 4).

DISCUSSION

Apple cider vinegar production with different techniques significantly affected pH and total acidity; vinegars produced with the submersion method had higher acidity. Acidity results of samples were in accordance with the study conducted by Hill et al.;[25] they found that apple vinegar samples had pH 2.9−5.7 and 10.4−105.7 g acetic acid/L. Saiz-Abajo et al.[26] reported that red grape wine vinegar samples had 10.00−110.91 g acetic acid/L total acidity and 1.30−17.6 g/L total solids. Water-soluble solids of traditional balsamic vinegars and wine vinegars were 69.0−75.5%[27] and 2−7%,[8] respectively. Total soluble solids, total ash, and total acidity of traditional balsamic vinegar samples were 55.5−66.7, 0.675−0.859, and 4.5−5%,[28,29] respectively. These results in the literature were in agreement with our results. It is well-known that red grape is an appropriate raw material type for vinegar production; the results showed that 'Red Delicious' apple is also suitable for vinegar production.

Phenolic compounds are believed to account for a major part of the antioxidant capacity in many plants and foods.[30] Ninfali et al.[31] reported that the total phenolic content of apple vinegar was 202 mg/L; total phenolics of apple cider vinegars in their study were higher than that of apple cider vinegars in our study, maybe due to apple varieties and vinegar production methods. Budak and Guzel-Seydim[17] reported that ORAC, TEAC, and total phenolic content, of traditional wine vinegar had 10.50 μmol/mL TE, 13.50 mmol/L TEAC, and 2690 mg L^{-1} GAE; industrial wine vinegar had 8.84 μmol mL^{-1} TE, 10.37 mmol/L TEAC, and 2461 mg L^{-1} GAE total phenolic content, respectively; in our study apple cider vinegar had lower total antioxidant activity. Total phenolic content varies according to the type of vinegar. Total phenolic content of the persimmon vinegar made from persimmon Saijyo varieties was 799 μg of gallic acid equiv/mL, followed by 733 μg of gallic acid equiv/mL for unpolished rice vinegar and 452 μg of gallic acid equiv/mL for persimmon vinegar made from Hiratanenashi varieties.[32] Ubeda et al.[33] indicated that total phenolic contents of persimmon vinegars changed between 268.0 and 397.5 mg GAE/kg. Total phenolic contents of red wine vinegars produced in barrels made from different woods were between 1006.19 and 1882.7 mg/L.[34] In our study, total phenolic contents of apple cider vinegars were similar to the other types of vinegars stated in the literature. Apple has significant total antioxidant activity due to its content of quercetin glycosides, epicatechin, chlorogenic acid, procyanidins, dihydrochalones, and hydroxycinnamic acid derivatives.[35,36] Chlorogenic acid is the most abundant hydroxycinnamic acid present in apple juice. Moreover, it is one of the main phenolic compounds in apple ciders along with epicatechin and procyanidin.[37] In this study, VSTM vinegar had the highest contents of total phenolic and chlorogenic acid. Total phenolic and chlorogenic acid contents had generally similar tendencyies in apple vinegar samples in our study.

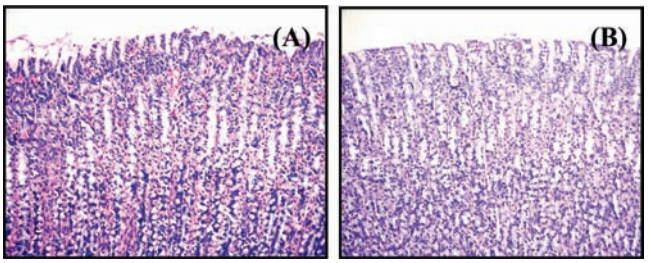

Figure 4. Microscopic views of transverse sections of stomach in normal rats (A) and in rats with the addition of apple cider vinegar (B) (100×).

Wine vinegars usually have high contents of polyphenolics, which are gallic acid, epicatechin, catechin, and caffeic acid,[38,17] whereas apple cider vinegar has a high amount of chlorogenic acid and relatively low amounts of gallic acid, epicatechin, and catechin (Table 4). A protective effect of chlorogenic acid against cardiovascular diseases with inhibition of LDL oxidation was reported.[12] Apple cider vinegar produced with a surface method including maceration (VSTM) had the highest total phenolic content, ORAC, and TEAC activities along with the highest content of chlorogenic acid.

In this study a high-cholesterol diet resulted in hypercholesterolemia in rats, and blood triglyceride and total cholesterol levels were increased compared with the CNT group. Levels of triglyceride decreased at different levels and approximated CNT group levels in all apple cider vinegar groups. Regardless of the production method, apple cider vinegars decreased triglyceride levels of rats; especially, triglyceride results of VST group was similar to CNT group. Similar to our results, Miceli et al.[39] reported that consumption of *Citrus bergamia* juice for 30 days in Wistar rats fed a hypercholesterolemic diet provided a significant reduction in serum levels of triglycerides and an increase in HDL levels. Nalini and Kapoor[40] also reported the effect of plant fruits (gall nut, bedda nut, and gooseberry) in increasing HDL

cholesterol according to a hypercholesterolemia-inducing diet. HDL is known as good cholesterol because it carries the cholesterol and cholesterol ester from peripheral tissues to the liver for catabolism.[41] Nofer et al.[42] reported that the increase in HDL may slow the atherosclerosis process. Our results showed that apple cider vinegar treatment increased HDL level when compared with CHOLCNT and CNT groups. Polyphenolics have been claimed to play an important role in affecting human health by preventing several diseases including cancer, hypertension, heart attack, and diabetes;[6] apple cider vinegar is also rich in polyphenolics such as chlorogenic acid, gallic acid, catechin, epicatechin, caffeic acid, and *p*-coumaric acid. Apple cider vinegar produced with the surface method including maceration (VSTM) had the highest contents of total phenolics and chlorogenic acid and ORAC and TEAC activities; the animal study showed that the VSTM group had the highest HDL and lowest LDL levels.

Miceli et al.[39] concluded that *C. bergamia* juice consumption by rats affected the hepatic triglyceride synthesis and activity of lipoprotein lipase; we also assume that constituents of apple cider vinegar might have an effect on these processes. Positive health effects of vinegars have been claimed in recent years such as inhibition of DNA damage,[11] protective effect against cardiovascular diseases with inhibition of LDL oxidation,[12] increased intestinal calcium absorption,[13] reduced blood pressure,[14] positive effects on blood glucose response and serum insulin,[15] and potential antitumor effect of apple cider vinegars has been noted.[16]

Similar to our results, Sudhahar et al.[43] reported that a high-cholesterol diet increased the hepatic enzyme ALP, AST, and ALT levels in Wistar rats; favorably, administration of lupeol and lupeol linoleate, which are present in various fruits and vegetables, reduced the levels to near control in their study. Hypercholesterolemia promotes ischemic tissue damage.[44] The activities of hepatic enzymic markers of cellular damage and inflammation such as ALP, ALT, and AST decreased in apple cider vinegar fed animals compared to high-cholesterol-fed animals (CHOLCNT) regardless of the production method. Lee et al.[45] reported that plasma AST activity was significantly reduced in the cinnamic acid and its synthetic derivative supplemented groups. Arafa[46] reported that the activities of serum AST and ALT were increased by more than 2- and 5-fold, respectively, in animals that were maintained on a high-cholesterol diet compared to the control animals.

The current study demonstrated that blood glucose levels were significantly higher than the control group in rats given apple cider vinegar along with a high-cholesterol diet in contrast to results of Ebihara and Nakajima,[15] Liljeberg et al.,[47] and Brighenti et al.,[48] suggesting that glucose levels of vinegar-administered healthy volunteers decreased 30%; vinegar and its acetic acid component have been suggested to be the factors resulting in delayed gastric emptying. In our study, 12 h fasted blood glucose was evaluated, not the postprandial blood glucose level. Further study is needed to clarify the effect of apple cider vinegar consumption on blood glucose.

Our results showed that a high-cholesterol diet resulted in hepatic steatosis. Histopathological results showed fat accumulation in liver in hypercholesterolemic rat groups when compared to control groups; a similar conclusion was also reported by Ziaee et al.[23] Apple cider vinegars produced using the submersion method with or without maceration (VSBM and VSB groups) significantly decreased steatosis. The histopathological analysis of the liver supported the biochemical data and indicated a protective effect of apple cider vinegar consumption on the development of liver damage due to a hypercholesterolemic diet. Miceli et al.[39] and Fki et al.[49] concluded that the histopatological analyses of the liver indicated affirmative results against fat accumulation in rats fed a cholesterol-enriched diet with olive mill wastewater extract or *C. bergamia* juice. Miceli et al.[39] observed a protection of hepatic parenchyma in histopathological analysis of *C. bergamia*-administered rats; in fact, hepatocytes of medium and small caliber, and not confluent vesicles, were observed.

During 7 weeks the increase in body weight was not significant in groups fed apple cider vinegars produced without the maceration process; this result is important because the control group, which did not receive any cholesterol administration, had significant body weight increase.

This is the first report confirming that apple cider vinegar had affirmative effects on blood lipid levels, liver functions and steatosis, and body weight increase. Further studies are needed to clarify the mechanism of apple cider vinegars on metabolism.

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