Fruit vinegars attenuate cardiac injury via anti-inflammatory and anti-adiposity actions in high-fat diet-induced obese rats

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ABSTRACT

Context: Fruit vinegars (FVs) are used in Mediterranean folk medicine for their hypolipidemic and weight-reducing properties.

Objective: To investigate the preventive effects of three types of FV, commonly available in Algeria, namely prickly pear [Opuntia ficus-indica (L.) Mill (Cactaceae)], pomegranate [Punica granatum L. (Punicaceae)], and apple [Malus domestica Borkh. (Rosaceae)], against obesity-induced cardiomyopathy and its underlying mechanisms.

Materials and methods: Seventy-two male Wistar rats were equally divided into 12 groups. The first group served as normal control (distilled water, 7 mL/kg bw), and the remaining groups were respectively treated with distilled water (7 mL/kg bw) and vinegars of pomegranate, apple or prickly pear (at doses of 3.5, 7 and 14 mL/kg bw, acetic acid content as mentioned above) along with a high-fat diet (HFD). The effects of the oral administration of FV for 18 weeks on the body and visceral adipose tissue (VAT) weights, plasma inflammatory and cardiac enzymes biomarkers, and in heart tissue were evaluated.

Results: Vinegars treatments significantly (p < .05) attenuated the HFD-induced increase in bw (0.2–0.5-fold) and VAT mass (0.7–1.8-fold), as well as increase in plasma levels of CRP (0.1–0.3-fold), fibrinogen (0.2–0.3-fold), leptin (1.7–3.7-fold), TNF-α (0.1–0.6-fold), AST (0.9–1.4-fold), CK-MB (0.3–1.4-fold) and LDH (2.7–6.7-fold). Moreover, vinegar treatments preserved myocardial architecture and attenuated cardiac fibrosis.

Discussion and conclusion: These findings suggest that pomegranate, apple and prickly pear vinegars may prevent HFD-induced obesity and obesity-related cardiac complications, and that this prevention may result from the potent anti-inflammatory and anti-adiposity properties of these vinegars.

Introduction

The prevalence of obesity in the world has risen dramatically and became a global-health public challenge (Crino et al. 2015). Obesity is the most important risk factor for the development of type-2 diabetes, hyperlipidemia, hypertension and cardiovascular disease (CVD) (Wisse et al. 2007). Several lines of evidence indicate that obesity is strongly associated with structural and functional changes in the heart in both human and animal models, now referred as ‘obesity cardiomyopathy’ (OC) (Wong & Marwick 2007). Mechanisms contributing to OC could include: altered cardiac metabolism, inflammation, oxidative stress, excess visceral adiposity, cardiac fibrosis and hypertrophy (Aurigemma et al. 2013; Cavalera et al. 2014). Among these pathophysiological mechanisms, hyperlipidemia-induced visceral adipose tissue (VAT) accumulation and inflammation are the upstream indicators in the cascade and have emerged as crucial factors in obesity-induced cardiac remodeling and dysfunction (Bays et al. 2008; Wang & Nakayama 2010).

In Framingham heart study, the incidence of the myocardial infarction and stroke was significantly associated with visceral adiposity (Lee et al. 2010). Visceral fat promotes OC through the active production of numerous immunomodulatory factors that affect the cardiovascular system both directly and indirectly (Lyon et al. 2003; Trayhurn & Wood 2004). In fact, tumor necrosis factor-α (TNF-α), leptin, as well as other circulating inflammatory biomarkers levels increase with obesity, and correlate strongly with percent abdominal fat in males and females (Westphal 2008). TNF-α has been proved to stimulate cardiomyocyte apoptosis in various cardiac diseases, decrease cardiac contractility in vitro, induce mitochondrial oxidative stress and cause cardiac dysfunction (Bajaj & Sharma 2006; Mariappan et al. 2007; Gatta et al. 2012). TNF-α has also been shown to be a mediator of hypertrophy contributing to adverse left ventricular remodeling (Del Re et al. 2010). Leptin was shown to enhance arterial thrombosis and platelet aggregation (Sierra-Johnson et al. 2007). Moreover, leptin was reported to promote cardiomyocyte hypertrophy in vitro, induce cardiac fibrosis and exacerbate coronary endothelial dysfunction (Korda et al. 2008; Leifheit-Nestler et al. 2013; Xie et al. 2015). In contrast, visceral fat might have beneficial metabolic effects by producing...
adiponectin, which epidemiological and experimental studies supports a protective role in CVD (Ouchi et al. 2006; Fontana et al. 2007).

In addition, several inflammatory indicators [such as homocysteine, fibrinogen, white blood cell count and the C-reactive protein (CRP)] are highly correlated with the degree of OC (Zaldívar et al. 2006; Darvall et al. 2007; Wee et al. 2008; Uysal et al. 2014). Studies have shown that an elevated white blood cell count and plasma CRP levels are associated with the development of CVD (Twig et al. 2012; Razavi et al. 2013). However, a large body of evidence has established fibrinogen as independent risk factor for CVD, and as an important mediator through which traditional risk factors, such as low-density lipoprotein, exert their effects (Clark et al. 2012). On the other hand, elevated levels of homocysteine have been associated with increased risk of CVD (Hollender et al. 2006). Therefore, anti-adiposity and anti-inflammatory therapies appear to be promising approaches in dealing with OC. Fruits and fruit-based products containing several bioactive compounds with anti-adiposity and anti-inflammatory properties have been shown to confer a protection against obesity-induced cardiac disorders (Luis 2013; Siriwardhana et al. 2013; Joseph et al. 2016).

Fruit vinegar (FV) is a kind of beverage and is becoming more popular throughout the world (Liu et al. 2008). Previous findings showed that vinegar and acetic acid (the main ingredient of vinegar) affect lipid profile and weight loss (Kondo et al. 2009; Petsiou et al. 2014). Studies have shown that vinegar and acetic acid (the main ingredient of vinegar) affect lipid profile and weight loss (Kondo et al. 2009; Petsiou et al. 2014). Therefore, anti-adiposity and anti-inflammatory therapies appear to be promising approaches in dealing with OC. Fruits and fruit-based products containing several bioactive compounds with anti-adiposity and anti-inflammatory properties have been shown to confer a protection against obesity-induced cardiac disorders (Luis 2013; Siriwardhana et al. 2013; Joseph et al. 2016).

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Material and methods

Materials

All the vinegars (pomegranate, prickly pear and apple) used in this study were obtained from the Algerian National Office for Wine Products Trading (ANOWPT, Algiers, Algeria). Chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of the high-fat diet

The normal diet which is commercially obtained from ONAB, Algiers, had the following composition: 22% crude protein, 4.2% crude oil, 9% humidity and 1–2% vitamins and minerals.

The normal pelleted diet was powdered and modified by adding 30.8% (w/w) melted cow abdominal fat, and 10% (w/w) corn starch. Then, the powdered diet was pelleted and regarded as high-fat diet (HFD). It was modified from the methods reported by Charradi et al. (2011) and Lee et al. (2013a). Normal chow diet (ND) contains 10% of Kcal as fat with an energy density of 3.85 Kcal/g, while HFD contains 45% of Kcal as fat with an energy density of 4.75 Kcal/g.

Animals

All research and animal care procedures were approved by the Institutional Animal Care Committee of the National Administration of Algerian Higher Education and Scientific Research. Ethical approval number: 981-1 law of 22 August 1998. A total of 127 male Wistar rats (7–8 weeks old; 210–230 g; Pasteur Institute, Algeria) were used for the study. Rats were single-housed in a temperature-controlled room (25 ± 1 °C), with relative humidity of 45–53% and 12:12 h light/dark cycle. Water and normal chow diet were provided ad libitum. All animals were acclimatized for 7 d before starting the experiments.

Acute toxicity study

To study acute toxicity in rats (Suganthi et al. 2014), overnight fasted animals were randomly divided into four groups with five rats in each group, avoiding any inter-group differences in body weight. The control group was given distilled water, and the experimental groups were given vinegars of pomegranate, prickly pear or apple at the dose of 2000 mg/kg bw, using an oral gavage. The animals were observed individually for signs of acute-toxicity and behavioural changes for 3 h post-dosing, and at least once daily for 14 days.

30-Day subchronic toxicity study

The experiment was conducted according to the protocols described by Golafkhhrabadi et al. (2014) with minimum modification: 35 rats were randomly divided into seven groups (n = 5), a control group and six treatment groups. The vinegars of pomegranate, prickly pear and apple were administered orally on a daily basis for 30 days at single doses of 2500 and 5000 mg/kg bw, while the control group received only distilled water. All rats were observed at least twice daily for morbidity or mortality, changes in posture, changes in skin, fur, eyes, mucous membranes and behaviours. Body weights of the animals were measured every three days. On day 31, rats were anesthetized by intraperitoneal injection of urethane (1 g/kg bw); after blood collection by cardiac puncture in a heparin tube, the rats were sacrificed and then dissected. Necropsy of all animals was carried and organ weights (heart, liver, kidney and spleen) were recorded separately and expressed as relative weight (organ weight/100 g of bw). Plasma chemistry was assessed by the methods described in the ‘Hematological and biochemical analysis’ section. Parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (γGT), glucose (GLU), urea, creatinine (CREA) and creatine kinase (CK).

Experimental design

According to the protocol described in Figure 1, seventy-two rats were divided into 12 groups (n = 6), one group was fed an ND,
while the rest were fed an HFD. Rats fed on ND were adminis-
tered orally with distilled water (7 mL/kg/d), whereas rats fed on
HFD groups received an oral gavage of either distilled water
(7 mL/kg/d), acetic acid (7 mL/kg/d) or the vinegar of pomegran-
ate (PV), prickly pear (PPV) or apple (AV) at doses of 3.5, 7, or
14 mL/kg/d. Prior to use, the acetic acid concentration of the FVs
was adjusted to be equivalent to that of the control treatment
(acetic acid), i.e., 0.5% (w/v). Food consumption was measured
every 3 days, and body weight was determined once a week.

At the end of the experimental period (18 weeks), rats were
anesthetized by urethane (1 g/kg bw, i.p.), euthanized, and then
blood samples were collected for hematological and biochemical
analysis. The heart was excised aseptically and weighed immedi-
ately. VATs (perirenal, mesenteric and epididymal) were col-
lected and weighed, followed by immediate freezing in liquid
gas nitrogen and stored at -80°C for further analysis.

**Hematological and biochemical analysis**

Leukocyte count was immediately performed after sample col-
lection using a hematology analyzer (PCE 210, ERMA Inc, Tokyo,
Japan). Plasma total cholesterol (TC), triglycerides (TG), high-
density apolipoprotein-cholesterol (HDL-c), GLU, AST, ALT,
γGT, CK, creatine kinase-MB isoenzyme (CK-MB), urea, CREA,
lactate dehydrogenase (LDH) and CRP concentrations were
measured using Roche Hitachi 902 auto analyzer (Roche
Molecular Systems, Branchburg, NJ). Plasma low-density
lipoprotein cholesterol (LDL-c) concentration were determined
according to the formula described by Friedewald et al. (1972),
and the coronary risk index (CRI) was calculated as TC/HDL-c
(Tzeng et al. 2012).

Plasma homocysteine concentrations were measured on an
IMMULITE 2000 XPI automated immunoassay analyzer
(Siemens, Flanders, NJ), and fibrinogen levels by SPINREACT
Kits (Spain) according to the manufacturer’s instructions. Plasma
leptin, adiponectin and TNF-α concentrations were determined
by the enzyme-linked immunosorbent assay (ELISA, DRG
Instruments GmbH, Marburg, Germany).

**Measurement of leptin, adiponectin, and TNF-α protein
levels in VAT**

The same ELISA assay kits were used to determine the immu-
noreactive cytokines from VAT after homogenization of 200 mg
of frozen tissue in 400 μL of RIPA buffer (0.625% Igepal CA-630,
0.625% sodium deoxycholate, 6.25 mM sodium phosphate and
1 mM ethylene-diamine-tetraacetic acid at pH 7.4) containing
10 μg/mL of a protease inhibitor cocktail (Sigma-Aldrich,
St. Louis, MO) as reported by Lira et al. (2010).

**Histopathological staining**

Excised hearts were fixed in 10% buffered formalin, and
embedded in paraffin. Serial 5 μm heart sections from each group

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**Figure 1.** Study flowchart explaining the various groups of rats and treatments. ND: normal chow diet; HFD: high-fat diet; Dw: distilled water; Ac: acetic acid; PV: pomegranate vinegar; PPV: prickly pear vinegar; AV: apple vinegar.
were stained with Masson’s trichrome, and hematoxylin and eosin (H&E). To evaluate the histopathological damage, each image of the sections was captured using a light microscope (400× amplification, Leica Store Miami, Coral Gables, FL) equipped with a digital camera system (Premiere camera, model MA88-500. Microscopes America Inc., Cumming, GA). Cross-sectional length of cardiomyocytes with centrally located nuclei (to ensure the same plane of sectioning) were measured using the NIH Image J software (Version 1.42, National Institutes of Health, MD) (Marin et al. 2011). The percentage of fibrosis was calculated with the histogram function of the Adobe Photoshop software (Adobe, San Jose, CA) as reported previously (Doser et al. 2009). Slides were examined by a pathologist who was not aware of the grouping.

**Statistical analyses**

Data are expressed as the mean ± standard error of the mean (SEM). Significant differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by Fisher’s least significant difference (LSD) test for multiple comparisons using GraphPad Prism version 6.01 (GraphPad Software Inc, San Diego, CA). Difference was considered to be significant at p < .05.

**Results**

**Acute oral toxicity study**

During the acute oral toxicity experiments, oral administration of the tested vinegars at a dose of 2000 mg/kg did not produce any signs of toxicity, and no death occurred.

**30-Day subchronic toxicity study**

Daily oral administration of vinegars produced from pomegranate, apple or prickly pear juices for 30 consecutive days did not induce any obvious symptom of toxicity in rat including for the highest dose tested of 5000 mg/kg bw daily. No lethality was recorded during the 30 days of vinegar administration. No difference in general behaviour, food and water consumption were observed between the groups (data not shown).

At the dose of 5000 mg/kg, PV reduced significantly the bw of rats between the 12th and 18th day of the treatment compared to the control group (p < .05) (Supplementary Figure 1). Furthermore, from the 21st day to the last day, all the tested vinegars at doses of 2500 and 5000 mg/kg significantly reduced rats’ bw (p < .05) (Supplementary Figure 1). There was no significant effect on relative weights of liver, heart, spleen and kidney of the treated group compared to the control rats (Supplementary Table 1). No treatment-related gross pathology was observed.

Plasma biochemical data at the end of the study are presented in Supplementary Table 1. No significant changes were observed in the clinical chemistry parameters (urea, CREA, AST, ALT, γGT and CK) measured between the treated and control groups (Supplementary Table 1). There was a significant reduction of glycemia after 30 days of treatment with PV at the dose of 5000 mg/kg when compared to the control group animals (p < .05) (Supplementary Table 1).

**Effect of FVs on body weight and food intake**

As shown in Table 1, the body weights of HFD-fed rats in the FV-treated, acetic acid-treated, and vehicle-treated groups were
monitored over the 18-week treatment period. At the end of treatment, the bw of FV-treated rats was significantly lower than the rats in the vehicle-treated group \((p < .05)\). FVs significantly prevented the bw gain at a low dose \((3.5 \text{ mL/kg/d})\), a moderate dose \((7 \text{ mL/kg/d})\) and high dose \((14 \text{ mL/kg/d}; p < .05)\). Similar results were observed in rats treated with acetic acid. In particular, the groups treated with PV had more efficient control of bw increase in a dose-dependent manner compared to that of the groups treated with PPV or AV. No significant differences in daily food intake were observed among the groups over the experimental period.

**Effect of FVs on weight of heart and VAT**

The weight of heart and VAT from HFD-fed rats in the FV-treated, acetic acid-treated and vehicle-treated groups were assessed after the treatment period. Heart weight was significantly lower in FV-treated and acetic acid-treated groups than in their vehicle-treated counterparts \((p < .05)\) (Table 1). A significant reduction in VAT weight was observed in low dose \((3.5 \text{ mL/kg/d})\), moderate dose \((7 \text{ mL/kg/d})\), and high dose \((14 \text{ mL/kg/d})\) of FV-treated rats compared to control rats \((p < .05)\) (Table 1). The weight of mesenteric, epididymal and perirenal fat pads was significantly lower in FVs \((3.5, 7 \text{ or } 14 \text{ mL/kg/d})\) treated, and HFD-fed rats compared with vehicle-treated, HFD-fed rats \((p < .05)\) (Table 1). Similarly, after treatment with acetic acid, mesenteric and epididymal fat pads were 71.4%, and 4.5% lower, respectively, than in their vehicle-treated counterparts with a slightly higher perirenal fat pad (Table 1). AV showed more efficiency in this regard than the other vinegars.

**Effect of FVs on plasma lipids**

The HFD caused elevated concentrations of plasma TG, TC and LDL-c (Table 2). Rats that received FV treatments \((3.5, 7 \text{ or } 14 \text{ mL/kg/d})\) showed significantly decreased plasma TG, TC, and LDL-c concentrations compared with vehicle-treated, HFD-fed rats \((p < .05)\) (Table 2). Plasma TG, TC, and LDL-c concentrations were significantly decreased by 42%, 9.3%, and 34.9%, respectively, in acetic acid-treated, HFD-fed rats \((p < .05)\) (Table 2). PV was more effective in preventing HFD-induced lipid metabolism disorder when compared with the remaining ones.

The plasma concentration of HDL-c in HFD-fed rats was reduced to 20.1% of the level in the ND-fed group (Table 2). After 18 weeks of treatment with FVs \((3.5, 7 \text{ or } 14 \text{ mL/kg/d})\) or acetic acid, the plasma HDL-c concentration in HFD-fed rats was elevated to nearly that of the ND-fed rats, more particularly in rats treated with high-dose PV \((14 \text{ mL/kg/d})\).

The HFD-fed groups treated with FVs \((3.5, 7 \text{ or } 14 \text{ mL/kg/d})\) showed a significant and dose-dependent reduction in the CRI when compared with the values recorded for their vehicle-treated counterparts \((p < .05)\) (Table 2), and among the groups, those treated with PV exhibited the most reductions. Similarly, treatment with acetic acid significantly prevented the elevation of the TC/HDL-c ratio in HFD-fed rats \((p < .05)\) (Table 2.)

**Effect of FVs on plasma cardiac biomarkers**

Plasma levels of CK-MB, LDH and transaminases were significantly higher in HFD-fed rats than in ND-fed rats \((p < .05)\) (Table 2). Plasma levels of the above-mentioned enzymes were significantly decreased in FV-treated rats \((3.5, 7 \text{ or } 14 \text{ mL/kg/d})\) on an HFD than vehicle-treated rats on an HFD \((p < .05)\) (Table 2), and the degree of decrease was greater in PV-treated rats than those in either PPV- or AV-treated rats. Likewise, rats on an HFD treated with acetic acid showed a significant reduction in the plasma levels of CK-MB, LDH, AST and ALT by 30, 65, 42 and 27%, respectively, compared to vehicle-treated rats on an HFD \((p < .05)\) (Table 2).

**Effect of FVs on plasma inflammatory biomarkers**

HFD-fed rats showed significantly higher plasma levels of CRP, fibrinogen and homocysteine than ND-fed rats \((p < .05)\) (Table 2). Plasma levels of these inflammatory biomarkers in FV-treated, HFD-fed rats were significantly decreased, particularly in those treated with PV \((p < .05)\) (Table 2). Results observed in the FV-treated groups were similar to the HFD-fed rats treated with acetic acid (Table 2).

**Effect of FVs on total leukocyte count**

In the HFD-fed group, total leukocyte count was significantly higher compared with that of the ND-fed group \((p < .05)\), but was significantly reduced in FV-treated, HFD-fed groups \((p < .05)\) (Table 2). Similar results were also obtained after treatment with acetic acid. PV showed more efficiency in this regard than PPV or AV.

**Effect of FVs on plasma and VAT cytokine levels**

Plasma adiponectin level in HFD-fed rats was significantly lower than in ND-fed animals \((p < .05)\), but was significantly increased in FV-treated, HFD-fed rats \((p < .05)\) (Table 3), with the highest level observed in the group treated with \(14 \text{ mL/kg/d}\) PV (Table 3). The level of adiponectin in VAT of HFD-fed rats was significantly lower compared with that of the ND-fed rats \((p < .05)\), but its level was significantly higher in the VAT of HFD-fed rats treated with FVs \((p < .05)\) (Table 3), especially in the group treated with \(14 \text{ mL/kg/d}\) AV (Table 3). Similarly, rats treated with acetic acid showed a significant increase of adiponectin level in both plasma and VAT compared with vehicle-treated, HFD-fed rats \((p < .05)\) (Table 3).

Plasma levels of leptin and TNF-\(\alpha\) in HFD-fed rats were significantly higher than in ND-fed rats \((p < .05)\) (Table 3). The levels of plasma leptin and TNF-\(\alpha\) were significantly decreased in HFD-fed rats treated with FVs \((3.5, 7 \text{ or } 14 \text{ mL/kg/d})\), relative to those in vehicle-treated \((p < .05)\) (Table 3). Plasma leptin and TNF-\(\alpha\) levels in HFD-fed rats treated with acetic acid were 58% and 43%, respectively, lower than those in their vehicle-treated counterparts (Table 3). Leptin and TNF-\(\alpha\) levels were also significantly higher in VAT of HFD-fed rats compared to those of ND-fed rats \((p < .05)\) (Table 3). In contrast, the levels of leptin and TNF-\(\alpha\) were significantly decreased in VAT of HFD-fed rats treated with FVs \((3.5, 7, 14 \text{ mL/kg/d})\) compared with their vehicle-treated counterparts \((p < .05)\) (Table 3). Similar results were found in rats treated with acetic acid (Table 3). Compared to other treatment groups, those receiving moderate and high doses of PPV \((7 \text{ and } 14 \text{ mL/kg/d})\), respectively) had the lowest VAT and plasma leptin levels. However, those receiving PV at the same doses \((7 \text{ and } 14 \text{ mL/kg/d})\) had the lowest VAT and plasma TNF-\(\alpha\) levels.
Table 2. Effect of fruit vinegars (FVs) on lipids profile, leukocyte count, and plasma inflammatory and cardiac biomarkers.

| Parameter                      | Ctrl     | OB       | AA       | PV1      | PV2      | PV3      | PPV1     | PPV2     | PPV3     | AV1      | AV2      | AV3      |
|--------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| **Plasma lipids profile**      |          |          |          |          |          |          |          |          |          |          |          |          |
| TG (mg/dL)                     | 104.2 ± 2.8 | 172.2 ± 6.1 | 99.8 ± 2.6 | 103.2 ± 0.8 | 89.6 ± 2.4 | 76.5 ± 1.1 | 112.8 ± 2.8 | 93.3 ± 1.5 | 82.3 ± 2 | 106.3 ± 3.8 | 89.3 ± 3.6 | 79.1 ± 3.4 |
| TC (mg/dL)                     | 61.9 ± 1.7 | 75.2 ± 2.6 | 68.1 ± 1.9 | 66.9 ± 0.5 | 65.1 ± 1.7 | 63.3 ± 0.9 | 69.1 ± 1.7 | 68.1 ± 1.1 | 63.3 ± 1.6 | 67.2 ± 2.4 | 66.3 ± 2.7 | 64.7 ± 1.6 |
| HDL (mg/dL)                    | 50.7 ± 1.3 | 40.5 ± 1.4 | 43.2 ± 1.1 | 50.2 ± 0.4 | 52.8 ± 1.4 | 55.4 ± 0.8 | 41.4 ± 1 | 43.6 ± 0.7 | 43.1 ± 1 | 47.6 ± 1.7 | 50.8 ± 2.1 | 51.5 ± 1.2 |
| LDL (mg/dL)                    | 32 ± 0.8 | 68.9 ± 2.4 | 44.8 ± 1.2 | 37.4 ± 0.2 | 30.8 ± 0.8 | 23.2 ± 0.3 | 50.2 ± 1.2 | 43.1 ± 0.7 | 41.6 ± 1 | 40.7 ± 1.4 | 33.3 ± 1.3 | 29 ± 0.8 |
| ALT (U/L)                      | 1.2 ± 0.0 | 1.8 ± 0.0 | 1.5 ± 0.0 | 1.3 ± 0.0 | 1.2 ± 0.0 | 1.6 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.4 ± 0.0 | 1.3 ± 0.0 | 1.2 ± 0.0 |          |
| **Plasma inflammatory biomarkers** |          |          |          |          |          |          |          |          |          |          |          |          |
| Fibrinogen (g/L)               | 2.9 ± 0.4 | 3.4 ± 0.4 | 2.8 ± 0.7 | 2.8 ± 0.1 | 2.8 ± 0.5 | 2.5 ± 0.6 | 3.0 ± 0.7 | 2.8 ± 0.4 | 2.7 ± 0.6 | 2.7 ± 1 | 2.6 ± 1 | 2.6 ± 0.6 |
| Blood leukocyte count           |          |          |          |          |          |          |          |          |          |          |          |          |
| White blood cells (×10^3/µL)    | 6.5 ± 0.1 | 15.1 ± 0.5 | 13 ± 0.3 | 8.5 ± 0.0 | 7.4 ± 0.2 | 5.2 ± 0.0 | 9.3 ± 0.2 | 8.1 ± 0.1 | 7.9 ± 0.1 | 10.8 ± 0.3 | 8.5 ± 0.3 | 6.4 ± 0.1 |
| Lymphocyte (×10^3/µL)          | 3.5 ± 0.1 | 9.3 ± 0.3 | 7.5 ± 0.2 | 6.1 ± 0.0 | 3.8 ± 0.1 | 3.1 ± 0.0 | 4.8 ± 0.1 | 4.8 ± 0.0 | 5.1 ± 0 | 6.8 ± 0.2 | 5.2 ± 0.2 | 2.4 ± 0.0 |
| Monocyte (×10^3/µL)            | 0.9 ± 0.0 | 1.7 ± 0.0 | 1.9 ± 0.0 | 1.2 ± 0.0 | 1.0 ± 0.0 | 0.8 ± 0.0 | 1.2 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0 | 2.2 ± 0.0 | 1.4 ± 0.0 | 0.5 ± 0.0 |
| Granulocyte (×10^3/µL)         | 2.1 ± 0.0 | 4 ± 0.1 | 3.5 ± 0.0 | 1.3 ± 0.0 | 2.4 ± 0.0 | 1.3 ± 0.0 | 3.2 ± 0.0 | 2.0 ± 0.1 | 1.7 ± 0.0 | 1.8 ± 0.0 | 1.9 ± 0.0 | 3.5 ± 0.0 |

Results are expressed as mean ± SEM, n = 6. Values are statistically significant at:
- p < .05, compared with OB group values.
- p < .01, compared with OB group values.
- p < .001, compared with OB group values.
- p < .05, compared with control group values.
- p < .01, compared with control group values.
- p < .001, compared with control group values.

Table 3. Effect of fruit vinegars (FVs) on plasma and visceral adipose tissue-derived (VAT) cytokines.

| Parameter                      | Ctrl     | OB       | AA       | PV1      | PV2      | PV3      | PPV1     | PPV2     | PPV3     | AV1      | AV2      | AV3      |
|--------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| **Adiponectin (ng/mL)**        | 78.3 ± 4.0 | 62.8 ± 4.4 | 83.6 ± 4.6 | 56.2 ± 0.9 | 90.5 ± 4.8 | 108.8 ± 3.2 | 88.8 ± 4.5 | 89.3 ± 2.9 | 96.4 ± 4.7 | 77.1 ± 5.8 | 78.5 ± 7.0 | 82.1 ± 4.9 |
| **Leptin (ng/mL)**             | 13.4 ± 0.6 | 38.1 ± 2.7 | 16.8 ± 0.9 | 13.1 ± 0.2 | 12.5 ± 0.6 | 8.5 ± 0.2 | 13.9 ± 0.7 | 8.4 ± 0.2 | 8.0 ± 0.3 | 13.9 ± 1.0 | 11.3 ± 1.0 | 8.4 ± 0.4 |
| **TNF-α (ng/mL)**              | 591.3 ± 30.5 | 996.1 ± 70.4 | 596.6 ± 32.2 | 572.1 ± 9.1 | 486.3 ± 25.8 | 395.8 ± 11.7 | 866 ± 43.9 | 755.5 ± 25.4 | 654.9 ± 31.9 | 702.1 ± 33.1 | 611.9 ± 54.8 | 613.8 ± 29.9 |
| **Adiponectin (ng/mg)**        | 364.9 ± 18.8 | 311.6 ± 22.8 | 375.8 ± 20.8 | 376.9 ± 6.0 | 386.7 ± 20.9 | 424.6 ± 12.6 | 377.9 ± 6.0 | 412.6 ± 13.8 | 415.4 ± 20.2 | 421.2 ± 31.8 | 420 ± 37.7 | 481.4 ± 23.5 |
| **Leptin (ng/mg)**             | 67.7 ± 3.5 | 92.5 ± 6.5 | 38.2 ± 2.0 | 30.4 ± 0.4 | 22.4 ± 1.1 | 13.1 ± 0.3 | 32.4 ± 1.6 | 13 ± 0.4 | 12.4 ± 0.6 | 32.1 ± 2.4 | 23.0 ± 2.0 | 13 ± 0.6 |
| **TNF-α (ng/mg)**              | 840.5 ± 43.4 | 1577 ± 111.6 | 900 ± 48.5 | 681.2 ± 32.3 | 610.8 ± 32.3 | 608.5 ± 18.0 | 831.5 ± 42.2 | 783.7 ± 26.3 | 731.2 ± 35.7 | 775.1 ± 58.6 | 702.6 ± 63.0 | 704 ± 34.3 |

Results are expressed as mean ± SEM, n = 6. Values are statistically significant at:
- p < .05, compared with OB group values.
- p < .01, compared with OB group values.
- p < .001, compared with OB group values.
- p < .05, compared with control group values.
- p < .01, compared with control group values.
- p < .001, compared with control group values.
Effect of FVs on cardiac histopathology

As shown in Figure 2(A), the cardiac longitudinal section in the H&E staining exhibited well-organized myofibrils and disorganized fibres in the normal control rats and in HFD-fed hearts, respectively. FV or acetic acid treatments prevented the histopathological alterations in the myofibril organization of the HFD hearts (Figure 2(A)). Cardiac transverse section H&E staining showed that the cardiomyocyte diameter was increased in the HFD group, while acetic acid or FV administration attenuated the HFD-induced cardiomyocyte hypertrophy (Figure 2(B)). Cell size measurements from the transverse section confirmed a significant reduction in the cardiomyocyte hypertrophy by FVs (3.5, 7 or 14 mL/kg/d) and acetic acid ($p < .05$) (Figure 2(C)).

Myocardial fibrosis was examined by Masson's trichrome staining. As shown in Figure 2(D), fibrosis was observed in the HFD-induced hearts. However, this fibrotic change was significantly attenuated in the FV and acetic acid-treated groups ($p < .05$) (Figure 2(D,E)). Quantitative fibrosis scoring of Masson's trichrome sections confirmed these observations (Figure 2(E)).

Discussion

Obesity is strongly associated with structural and functional changes in the heart in both human and animal models (Abel et al. 2008). Cardiac consequences of obesity include cardiac remodeling such as cardiac hypertrophy, cardiac fibrosis and subclinical impairment of left ventricle systolic and diastolic function (Qian et al. 2015). As observed in this current study, HFD feeding for 18 weeks significantly increased the rat body weight and hyperlipidemia. Under a HFD regimen, cardiac remodeling including cardiomyocyte disorganization, hypertrophy and fibrosis were evident in the hearts of obese rats. These were accompanied by significantly increased inflammatory markers levels and VAT mass. Our results are consistent with previous reports that the mechanisms underlying the obesity-induced cardiac remodeling are multifactorial using several mechanisms such as inflammation and excess accumulation of visceral fat (Bays et al. 2008; Wanahita et al. 2008; Dela Cruz & Matthay 2009; Wang & Nakayama 2010).

Vinegar has been used in a variety of chronic diseases. Growing evidence has suggested that vinegar can be used to prevent or treat obesity and/or obesity-related metabolic diseases.
(Cheong et al. 2014). In such abnormalities, apple cider vinegar can reverse insulin resistance, hyperglycemia and hyperlipidemia (Salbe et al. 2009; De Dios Lozano et al. 2012; Mitrou et al. 2015). However, grape vinegar can improve inflammatory factors that adversely affect coronary artery health (Setorki et al. 2011a).

A large body of evidence indicates that obesity is associated with a low-grade chronic inflammation characterized by increased amounts of pro-inflammatory cytokines and related factors, along with a high circulating leukocyte count (Ouchi et al. 2011; Chae et al. 2013; Esser et al. 2014). Here, we also found that there was increased plasma pro-inflammatory biomarkers and elevated blood leukocyte count in the obese rats, which matches with previous studies (Terra et al. 2009; Kim et al. 2013). These results suggested that the obesity causes inflammation, which promotes cardiomyopathy. Many previous studies have demonstrated a pivotal role of this pro-inflammatory state in the myocardial injury by directly inducing fibrosis, hypertrophy and ultimately contractility (Aukrust et al. 2004; Martínez-Martínez et al. 2014). Interestingly, all these abnormalities were prevented by treatments with FVs. The anti-inflammatory effect of FVs was closely associated with their cardioprotective effects. Furthermore, recent studies have reported that adiponectin has anti-inflammatory and anti-atherogenic properties, and suggest that high plasma adiponectin levels may be related to a lower risk of coronary heart disease (Nigro et al. 2014; Ohashi et al. 2014; Van Stijn et al. 2014). Our study showed that the FVs efficiently prevented decrease in plasma adiponectin level, which may explain their cardioprotective effects.

It has been recognized that, apart from overall obesity, the distribution of body fat can influence disease risk (Snijder et al. 2006). Thus, VAT accumulation is the fat depot most characterized as being associated with an increased risk of cardiovascular morbidity and mortality (Carmienke et al. 2013). Meanwhile, reduction in VAT has been shown to improve obesity-induced cardiomyopathy (Matsuzawa 2006). This improvement has been reported to be mainly due to the decrease in pro-inflammatory cytokines production (Malavazos et al. 2007). In the present study, TNF-α and leptin levels were higher, while adiponectin levels were lower in VAT of HFD-fed rats, indicating an obesity-induced systemic inflammation, and VAT dysfunction. Interestingly, FV treatments prevented HFD-induced VAT accumulation, and systemic imbalance between pro- and anti-inflammatory cytokines. It is speculated that systemic anti-inflammatory properties of FVs could result from the higher levels of adiponectin found in treated rats.

Finally, besides their cardiac effects, VAT-derived pro-inflammatory cytokines, like TNF-α and leptin, play an important role in adipocyte physiology (Cawthorn & Sethi 2008; Harris 2014). The high amount of leptin secreted by adipocytes (e.g., obesity), accounts for adipocyte differentiation, and increases lipolysis and β-oxidation (Yeaman 2004; Ewaschuk et al. 2014), on one side, and high secretion of pro-inflammatory cytokines in the other (e.g., TNF-α and interleukin-6) (Rajagopal et al. 2012). However, the increased levels of TNF-α are implicated in the induction of atherogenic adipokines, such as plasminogen activator inhibitor-1 and interleukin-6, and the inhibition of the anti-atherogenic adipokine, adiponectin (Ahn et al. 2007), as well as increase in the rate of adipocyte apoptosis (Barry et al. 2008). In particular, the imbalance between pro- and anti-inflammatory adipokines might play an important role in the formation of a vicious circle of inflammatory exacerbated VAT state, proven to increase the risk of cardiac complications in obesity (Bays & Ballantyne 2006; Sharma & Staels 2007). Thus, additional studies are required to further determine why and how FVs differentially regulate the production of adipokines in VAT.

Conclusions
The findings of the current study demonstrated the defensive role of vinegars produced from pomegranate, prickly pear and apple juices, against inflammation, hypertrophy and fibrosis in obesity-induced heart injury. Although the reduced bw gain and plasma lipids increase may partially contribute to their beneficial effects in OC. The beneficial actions of these vinegars are closely related with their ability to maintain increased levels of adiponectin in the plasma and VAT. This clearly suggests that these vinegars could be used for therapeutic application in the treatment of obesity-related cardiac disorders. In addition, TNF-α and leptin, for their endocrine and paracrine action, mediate the hypertrophic and fibrotic effects in HFD-induced hearts, and entail additional VAT inflammation. These results may provide a deeper understanding of the mechanism and treatment of hyperlipidemia-induced cardiac injury and obesity-related disorders. Both the newly made PPV and AV seem to be less active than PV in terms of preventing obesity and associated cardiac complications.

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The authors declare that they have no competing interests.

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