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Effects of sugarcane vinegar supplementation on oxidative stress and weight reduction in hyperlipidaemic mice

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Abstract

The present work aimed to evaluate the efficacy of sugarcane vinegar supplementation in hyperlipidaemic mice. The mice were divided into six experimental groups: negative control, positive control (hyperlipidaemic), lovastatin, low-dose, medium-dose, and high-dose supplementation groups that received sugarcane vinegar via force-feeding. Body and organ weights of the mice were measured at the end of the study, and the collected blood was subjected to analysis of plasma lipid profile, plasma antioxidant enzymes, and other biochemical parameters for protection against oxidative stress. The results revealed that sugarcane vinegar supplementation significantly reduced body weight and improved plasma lipid profile of the mice. Although mice from the high-dose group showed a significant reduction in plasma triglyceride and plasma low-density lipoprotein-cholesterol, no changes in plasma total cholesterol and plasma high-density lipoprotein-cholesterol were observed. Supplementation of sugarcane vinegar in hyperlipidaemic mice neither reduced plasma glucose levels, inhibited plasma α-amylase activity, nor increased plasma lipase levels when compared with non-supplemented hyperlipidaemic mice. Oxidative stress was slightly improved in mice, in response to supplementation with sugarcane vinegar where all supplemented mice exhibited a significantly lower plasma malondialdehyde level, and the mice from the high-dose group exhibited a significantly higher plasma superoxide dismutase level when compared with the hyperlipidaemic group. As sugarcane vinegar reduced oxidative stress in mice, especially in mice supplemented with a high dosage of the vinegar, sugarcane vinegar could be a functional food with great potential for use in health-promoting beverages.

Introduction

Vinegar has been widely used as an acidic condiment since ancient times. Among the different types of vinegar, fruit vinegar is popularly consumed as a functional beverage in the modern days. One of the most commonly studied vinegars is apple cider vinegar. A previous study has shown that supplementation with apple cider vinegar in hypercholesterolaemic ovariectomised mice could effectively reduce oxidative stress (Nazıroğlu *et al.,* 2014). Sugarcane vinegar, with its own health-promoting properties, is a good alternative to apple cider vinegar. In addition to sugars, sugarcane juice contains amino acids, vitamins such as niacin and riboflavin, and minerals such as calcium, phosphorus, manganese, zinc, and iron (> 9 mg/kg) (Duarte-Almeida *et al.,* 2006; Xiong *et al.,* 2014). The high levels of organic acids in natural vinegar promote metabolism, aid body detoxification, and eliminate fatigues; whereas polysaccharides in the vinegar could help the body to restore cellular activities, and cleanse urinary tract and kidneys (Chen *et al.,* 2013; 2015).

Sugarcane vinegar is produced from sugarcane juice through the processes of alcoholic fermentation and ethanoic acid fermentation (Chen *et al.,* 2011; Zheng *et al.,* 2016a). Literature has shown that total organic acids (He *et al.,* 2017) and total polyphenol content (Chen *et al.,* 2015) in sugarcane vinegar are 3.65% and 132.08 μg/mL, respectively. The main

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organic acid component in sugarcane vinegar is acetic acid. The other detected organic acids in the vinegar are oxalic, tartaric, acetic, and succinic acids (Chen *et al.,* 2015). As one of the most abundant organic acids in vinegar-based beverages, the concentrations of acetic acid in sugarcane vinegar range from 8.16 to 13.65 mg/g dry weight (DW). The tartaric acid content of vinegar is similar to that of succinic acid (1.50 mg/g DW), while the oxalic acid content of vinegar is the lowest (0.29 - 0.36 mg/g DW).

Ten major phenolic compounds such as caffeic acid, chlorogenic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, apigenin, coumarin, kaempferol, luteolin, and vanillin have been detected in sugarcane vinegar-based beverages (He *et al.,* 2017). The levels of caffeic acid, chlorogenic acid, ferulic acid, coumarin, and vanillin in the vinegar-based beverage were relatively high (> 1.0 mg/L); whereas the levels of cinnamic acid, apigenin, kaempferol, and luteolin were relatively low (< 0.1 mg/L). In addition, the level of *p*-coumaric acid was 0.731 mg/L. A previous study has reported that sugarcane wine and sugarcane vinegar effectively eliminate DPPH and OH radicals (Zheng *et al.,* 2015). The data showed that the efficacy increased with a larger sample volume; and the rate of DPPH radical elimination was higher in the treatment groups than in standards such as ascorbic acid and gallic acid. In addition, the study showed that sugarcane vinegar effectively eliminated NO^{2-}, thus indicating that it is an effective chelating agent. Additionally, sugarcane vinegar has more antioxidant properties than sugarcane wine.

Previous studies have determined the health-promoting properties of sugarcane juice, sugarcane wine, and sugarcane vinegar extracts (Yan *et al.,* 2012). Characterisation of antioxidants in sugarcane vinegar (Lin *et al.,* 2017) and its processing (Kang *et al.,* 2016) has been performed. Although *in vitro* antioxidant activity assays mimicking the antioxidation in human intestine have been performed by Zheng *et al.* (2016b), there is limited information on serum lipid metabolism regulation and oxidative stress inhibition by sugarcane vinegar. Therefore, the present work aimed to investigate the protective effect of antioxidant components in raw sugarcane vinegar on plasma lipid profile, antioxidant enzymes, and other biochemical parameters using a hyperlipidaemic mouse model.

Materials and methods

Chemicals and reagents

Analytical chemicals such as sodium hydroxide, hydrochloric acid, and anhydrous ethanol were purchased from Sinopharm Chemical Regents Co. Ltd. (Shanghai, China), and HPLC-grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Shanghai, China). LC-MS grade formic acid was purchased from CNW (Beijing, China). Polyphenol standards including benzoic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, sinapic acid, apigenin, catechin, coumarin, kaempferol, and luteolin were purchased from Sigma-Aldrich (Shanghai, China). Ultrapure water was obtained using a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA).

Commercial biochemical analysis kits for the determination of plasma triglycerides (TG), plasma total cholesterol (TC), plasma high-density lipoprotein-cholesterol (HDL-c), plasma low-density lipoprotein-cholesterol (LDL-c), fasting plasma glucose, malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), nitric oxide synthase (NOS), α-amylase, and lipase were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Sample preparation

Raw sugarcane vinegar was prepared by fermentation of sugarcane juice using wine yeast. Fermentation was performed according to a patented procedure (Chen *et al.,* 2014) reported by Chen *et al.* (2015). The fermentation began with alcohol fermentation using brewing yeast (*Saccharomyces cerevisiae*) purchased from Angel Yeast Co. Ltd. (Hubei, China), followed by acetic acid fermentation using *Lactobacillus* spp. that were purchased from DINGFENG Brewing Technology Co. Ltd. (Shanxi, China). Following fermentation, the observed product was a bright and clear yellow-brown liquid with a cane aroma.

Quantification of phenolic compounds by liquid chromatography-mass spectrometry (LCMS)

A Waters ACQUITY Ultra Performance LC (UPLC) system equipped with a Waters 2996 photodiode array (PDA) detector, a single quadrupole MS detector, and Empower Software (Waters Technology Co. Ltd., Shanghai, China) was used for HPLC analysis of phenolic compounds in sugarcane vinegar. The separation of phenolic compounds was achieved using the 2.1 × 100 mm, 1.7 μm ACQUITY UPLC® HSS T3 column (Waters Technology Co. Ltd., Shanghai, China). The chromatographic conditions were established based on the method described by He *et al.* (2017), with some modifications. Column temperature was set at 40°C, while injection volume and flow rate were 2 μL and 0.21 mL/min,

respectively. Mobile phase A contained 0.1% formic acid, and mobile phase B consisted of an acetonitrile and 0.1% formic acid mixture. Gradient elution was performed as follows: 0 - 0.88 min, 5% B; 0.88 - 1.28 min, 5 - 22% B; 1.28 - 4.48 min, 22% B; 4.48 - 9.08 min, 22 - 45% B; 9.08 - 13.88 min, 45% B; 13.88 - 14.5 min, 45 - 5% B; and 14.5 - 15.4 min, 5% B.

Electrospray ionisation was performed in positive ion mode, scanned by multi-reaction monitoring (MRM), with a capillary voltage of 3.0 kV and an ion source temperature of 500°C. Argon gas was used; the desolvation air flow rate, cone-hole air flow rate, and collision air flow rate were 350, 150, and 2.2 L/h, respectively. The linearity of phenolic standards was high (R^2 of > 0.9), with recovery rates of 102 - 112%. Triplicate determinations were performed to quantify phenolic compounds.

Animal experimentation

Thirty-six male Kunming mice (4 - 5 weeks old; body weight 16 - 20 g) were purchased from the Guangxi Experimental Animal Centre (Guangxi Medical University, Guangxi, China). Ethical approval (GXAAS/AEEIF/00001) was obtained, and animal experiments were performed in accordance with the guidelines of the Animal Care and Use Committee (ACUC), Guangxi Academy of Agricultural Sciences, Nanning City, China. Mice were individually housed under controlled room temperature (24 - 26°C) with 12 h light/dark cycles, and had *ad libitum* access to standard laboratory feed and filtered tap water. The mice were acclimatised for 1 w prior to high-fat diet intake.

Following acclimatisation, the mice were randomly allocated into six groups (n = 6 in each group), which included a negative control group and five hyperlipidaemic groups, including the positive control group (in which the mice were hyperlipidaemic without sugarcane vinegar supplementation). The other four hyperlipidaemic groups consisted of three supplementation groups (low, moderate, and high doses of sugarcane vinegar) and a statin group. Non-hyperlipidaemic control mice (NC) were provided regular mouse chow, whereas hyperlipidaemic groups were induced by oral feeding a high-fat diet (mainly composed of 78.8% regular mouse chow, 10% lard, 10% yolk powder, 1% cholesterol, and 0.2% bile salt) for 4 w.

All mice in the supplementation groups were administered sugarcane vinegar for an additional 4 w. During the intervention, the mice in the positive control (PC) and statin (SG) groups were fed with only a high-fat diet and were orally gavaged with normal saline and 7.5 mg/mL lovastatin solution, respectively; while the mice in the low dose (LD), medium dose (MD), and high dose (HD) groups were force-fed with 6-times diluted (1.67 mL/kg), 3-times diluted (3.333 mL/kg BW), and undiluted vinegar (10.0 mL/kg BW), respectively. The doses of sugarcane vinegar provided to the experimental mice were five to 20 times higher than the average daily intake of a healthy individual (~0.5 mg/kg BW). Body weights (BW) of all mice were also measured once a week, especially for vinegar dose adjustment. Food intake of the mice was also determined. Additionally, the mice were observed for any abnormal physical conditions and behavioural changes.

At the end of the experiment (week 8), all mice were anaesthetised using diethyl ether after measuring the BW. The mice were euthanised via cervical dislocation. Blood was collected from all fasting mice in heparinised tubes through venepuncture and centrifuged at 4000 rpm (4°C) for 15 min. The organs including heart, liver, and kidneys were harvested, washed with cold normal saline, cleaned off the adhesive tissues (fascia and blood vessels), dried, weighed, and transferred into small beakers filled with ice. The organs were then cut into smaller pieces, and homogenised with 9 volumes of normal saline. Finally, the homogenate was centrifuged at 4000 rpm (4°C) for 15 min. The plasma and organ samples were stored at -80°C prior to biochemical analyses. All biochemical analyses were performed within 1 w of the collection of plasma and organs. The organ/body weight (BW) index of the mice was calculated based on the following equation:

$$\text{Organ/body weight index (\%)} = \text{weight of organ/body weight} \times 100$$

Where, the body and organ weights were expressed in grammes (g) (Rajeh *et al.*, 2012).

Determination of lipid profile and plasma and liver biochemical parameters

Plasma lipid profiles including TC, TG, HDL-c, and LDL-c, as well as the levels of plasma fasting glucose, α-amylase, lipase, MDA, GPx, SOD, and NOS were determined at the end of the experiment (week 8). The activities of antioxidant enzymes were determined using the analytical assay kits (ZhongSheng BeiKong Biotechnology Co. Ltd., Beijing, China). MDA, GPx, SOD, and NOS levels in the organ homogenates of all experimental mice were also determined. All the biochemical assays were performed using the respective assay kits according to the instructions provided by the manufacturers (ZhongSheng BeiKong Biotechnology Co. Ltd.,

Beijing, China), and analysed using a Hitachi 7020 automatic biochemistry analyser (Tokyo, Japan).

Statistical analysis

All data were expressed as mean ± standard error of the mean. Statistical analyses were performed based on the analysis of variance (ANOVA) using IBM SPSS statistics version 23 (Armonk, NY, USA). Multiple comparisons were performed based on the LSD *post hoc* test. Significance level was set at $p < 0.05$.

Results

Quantification of phenolic compounds by LCMS

Eleven phenolic compounds were detected in the sugarcane vinegar sample using LCMS (Table 1). Among the phenolic compounds, *p*-coumaric acid, benzoic acid, chlorogenic acid, and ferulic acid were majorly detected. In contrast, a low concentration of caffeic acid was detected in the sample. Flavonoids were also found to be the minor compounds found in sugarcane vinegar. As shown in Figure 1, the level of coumarin was the highest among the flavonoids detected in sugarcane vinegar, followed by luteolin, apigenin, and kaempferol. In addition, except for caffeic acid, the amounts of flavonoids in the sugarcane vinegar were far lower than the amounts of phenolic acids.

Figure 1 shows the total ion chromatogram of the sugarcane vinegar sample (over 20 peaks). However, most compounds could not be identified using a single quadrupole MS detector. Although *p*-coumaric acid, sinapic acid, and catechin were detected in the sample, these phenolic compounds were not quantified in the present work.

Effects of sugarcane vinegar on body weight gain and organ/body weight index

The BW and organ/BW indices of the experimental mice are shown in Table 2. The changes in BW were calculated based on the difference in final and baseline body weights. The results showed a slight increase in BW (2.18%) in mice from the PC group when compared with that in mice from the other groups. Mice from the supplementation groups showed a significant reduction ($p < 0.05$) in BW than the PC mice, whereas HD mice had the most weight loss. Weight loss was not significantly different between LD and PC mice ($p > 0.05$). In addition, SG mice exhibited a significantly higher weight gain than PC mice ($p < 0.05$). This observation was consistent with the findings of Seo *et al.* (2014) in their research on tomato vinegar supplementation in obese mice, where the treatment groups exhibited reduced weight gain. On the contrary, NC mice had the highest increment of BW (22.86%) when compared with the other mice, showing that NC mice exhibited a normal weight gain.

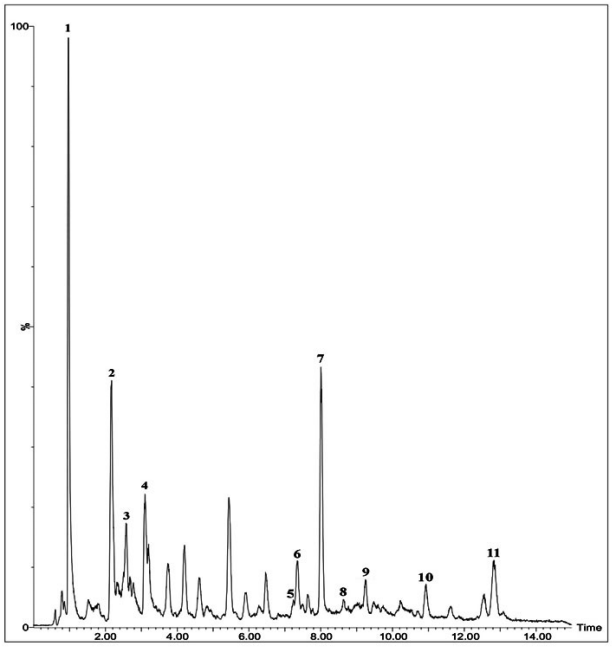

Figure 1. Total ion chromatogram of sugarcane vinegar sample.

Table 1. Phenolic contents in sugarcane vinegar.

Peak	Compounds	Retention time (min)	Molecular weight	$[M+H]^+$	Concentration (µg/L)
1	*p*-Coumaric acid	0.97	164.16	165.11	ND
2	Benzoic acid	2.17	122.12	123.05	1129.5 ± 87.3
3	Ferulic acid	3.10	194.18	195.12	1146.5 ± 18.2
4	Catechin	4.20	290.26	209.13	ND
5	Coumarin	7.24	146.14	147.07	17.6 ± 2.4
6	Caffeic acid	7.34	180.16	181.09	4.9 ± 0.4
7	Kaempferol	8.00	286.23	287.09	2.4 ± 0.1
8	Chlorogenic acid	8.62	354.31	355.07	1239.4 ± 9.3
9	Sinapic acid	9.24	224.21	225.07	ND
10	Luteolin	10.93	286.24	287.1	3.8 ± 0.06
11	Apigenin	12.82	270.24	271.1	2.8 ± 0.06

ND: not determined.

Table 2. Effect of sugarcane vinegar supplementation on changes in body weight and organ/body weight indices.

Group	Body weight changed (%)	Heart		Liver		Kidney	
		Weight (g)	Index (%)	Weight (g)	Index (%)	Weight (g)	Index (%)
NC	22.86 ± 0.93^{a}	0.18 ± 0.01^{a}	0.41 ± 0.03^{a}	1.89 ± 0.07^{a}	4.72 ± 0.13^{b}	0.36 ± 0.01^{a}	0.89 ± 0.03^{b}
PC	2.18 ± 3.14^{c}	0.16 ± 0.01^{ab}	0.42 ± 0.03^{a}	1.30 ± 0.06^{b}	5.36 ± 0.23^{a}	0.35 ± 0.02^{a}	1.02 ± 0.04^{a}
SG	10.39 ± 1.80^{b}	0.14 ± 0.01^{bc}	0.41 ± 0.02^{a}	1.07 ± 0.18^{b}	3.45 ± 0.20^{d}	0.28 ± 0.02^{b}	0.88 ± 0.04^{b}
HD	-14.69 ± 1.61^{e}	0.12 ± 0.01^{c}	0.45 ± 0.03^{b}	1.02 ± 0.10^{b}	3.32 ± 0.34^{d}	0.28 ± 0.01^{b}	0.88 ± 0.04^{b}
MD	-8.49 ± 2.34^{ed}	0.14 ± 0.01^{bc}	0.43 ± 0.02^{ab}	1.01 ± 0.07^{b}	3.44 ± 0.32^{d}	0.28 ± 0.01^{b}	0.93 ± 0.03^{b}
LD	-2.10 ± 3.09^{cd}	0.15 ± 0.01^{b}	0.42 ± 0.09^{a}	1.22 ± 0.09^{b}	3.97 ± 0.27^{c}	0.32 ± 0.02^{ab}	0.90 ± 0.06^{b}

Different lowercase superscript letters denote significant differences between groups ($p < 0.05$).

The results showed that except for the mice in the high-dose group, the heart/BW and kidney/BW indices of the experimental mice were not greatly affected by the sugarcane vinegar supplementation. A high-dose supplementation significantly increased the heart/BW index ($p < 0.05$); thus, it is hypothesised that sugarcane vinegar improves heart development. In contrast, the high-fat diet significantly increased the liver/BW and kidney/BW indices ($p < 0.05$). However, sugarcane vinegar supplementation significantly reduced these indices when compared with the control group ($p < 0.05$). Moreover, the liver/BW indices of the supplementation groups were significantly lower than those of the NC group ($p < 0.05$). In addition, the magnitudes of the reductions in MD and LD supplementations were smaller than those for HD supplementation.

Effect of sugarcane vinegar supplementation on lipid profiles

The efficacy of sugarcane vinegar can be observed based on the data obtained from the plasma lipid profile of the experimental mice. The lipid profiles of the experimental mice are shown in Table 3. High levels of plasma TG and TC were determined in the hyperlipidaemic mice, especially the PC mice. The results also showed that TG level of PC mice was significantly higher than that of NC mice ($p < 0.01$), which indicated that a successful high-fat diet-induced hyperlipidaemia. Meanwhile, the mice in the high-dose supplementation group had a significant reduction in plasma TG level when compared with PC mice ($p < 0.01$). HD mice also exhibited a significantly lower plasma TG levels than the MD and LD mice ($p < 0.05$). These findings demonstrated that consumption of an undiluted sugarcane vinegar beverage can reduce blood lipid levels, but not the blood cholesterol levels. Furthermore, no significant differences were found in plasma TC levels across all experimental groups ($p > 0.05$).

Table 3. Effect of sugarcane vinegar supplementation on lipid profile.

Group	Level (mmol/L)			
	TG	TC	LDL-c	HDL-c
NC	1.11 ± 0.17^{b}	2.64 ± 0.21^{a}	0.09 ± 0.03^{b}	1.87 ± 0.12^{b}
PC	2.23 ± 0.11^{a}	3.22 ± 0.30^{a}	0.24 ± 0.04^{a}	5.42 ± 0.50^{a}
SG	1.11 ± 0.14^{b}	2.72 ± 0.08^{a}	0.13 ± 0.03^{b}	5.11 ± 0.20^{a}
HD	1.21 ± 0.19^{b}	3.12 ± 0.23^{a}	0.12 ± 0.12^{b}	5.72 ± 0.30^{a}
MD	1.83 ± 0.20^{a}	3.17 ± 0.17^{a}	0.14 ± 0.03^{b}	5.66 ± 0.48^{a}
LD	1.81 ± 0.17^{a}	2.80 ± 0.18^{a}	0.07 ± 0.02^{b}	5.36 ± 0.33^{a}

Different lowercase superscript letters denote significant differences between groups ($p < 0.05$).

High-fat diet significantly increased the plasma LDL-c and HDL-c ($p < 0.01$) in the experimental mice when compared with the NC mice. In contrast, sugarcane vinegar supplementation significantly reduced the plasma LDL-c levels of the supplemented mice when compared with those of the PC mice ($p < 0.05$). As observed, high-dose supplementation did not significantly reduce the plasma HDL-c and LDL-c levels when compared with the lower dosages ($p > 0.05$). In brief, 4-week administration of sugarcane vinegar improved the lipid profile of the hyperlipidaemic mice; therefore, vinegar intake helps prevent high-fat diet-induced hyperlipidaemia, but not hypercholesterolaemia.

Effect of sugarcane vinegar supplementation on plasma glucose, α-amylase, and lipase levels

Besides the hypolipidaemic effect, sugarcane vinegar supplementation had minimal effect on plasma sugar levels. High-fat diet (PC group) caused a significant reduction in the plasma glucose levels and a significant increase in α-amylase and lipase activities when compared with normal chow (the NC group) ($p < 0.05$). As shown in Table 4, all mice that were supplemented with sugarcane vinegar showed no significant reduction in the levels of plasma

glucose and α-amylase when compared with the non-supplemented mice (PC group) ($p > 0.05$). This shows that organic acids such as oxalic, tartaric, acetic, succinic acids, and phytochemicals in sugarcane vinegar failed to reduce the blood sugar levels. Among the different doses of vinegar supplementation, low-dose supplementation was able to reduce the plasma glucose levels than medium- and high-dose supplementation. However, vinegar supplementation did not significantly reduce the plasma lipase activity ($p > 0.05$). In addition, no significant differences were found in the plasma lipase activities across the different vinegar dosages ($p > 0.05$).

Table 4. Effect of sugarcane vinegar supplementation on plasma glucose, α-amylase, and lipase levels.

Group	Glucose	α-Amylase (U/L)	Lipase (U/L)
NC	5.42 ± 0.08^{a}	3423.33 ± 173.21^{d}	38.67 ± 1.28^{b}
PC	2.32 ± 0.37^{bdc}	5027.33 ± 212.06^{ac}	66.00 ± 8.42^{a}
SG	1.86 ± 0.23^{c}	4509.67 ± 269.64^{bc}	63.00 ± 5.07^{a}
HD	3.02 ± 0.51^{b}	4821.33 ± 70.71^{ab}	59.00 ± 1.90^{a}
MD	2.03 ± 0.27^{bc}	4812.00 ± 128.37^{ab}	57.33 ± 1.69^{a}
LD	1.98 ± 0.25^{cd}	5131.00 ± 264.85^{a}	58.33 ± 2.74^{a}

Different lowercase superscript letters denote significant differences between groups ($p < 0.05$).

Effect of sugarcane vinegar supplementation on oxidative stress markers

Oxidative stress markers were determined to confirm the efficacy of sugarcane vinegar as a functional food for reducing oxidative stress. As shown in Figure 2 and Figure 3, the levels of plasma and organ MDA, GPx, SOD, and NOS were determined for all experimental mice. Mice with high-fat diet-induced hyperlipidaemia (PC) had a significantly lower plasma GPx level ($p < 0.01$) than the NC mice, whereas the plasma MDA level was significantly increased in the hyperlipidaemic mice ($p < 0.05$). Except for the plasma MDA level of MD group, no significant differences were observed between the mice supplemented with different dosages of sugarcane vinegar ($p > 0.05$) with respect to the levels of all oxidative stress markers. Moreover, the vinegar-supplemented mice had a significantly higher plasma SOD activity than the non-supplemented hyperlipidaemic mice (PC) ($p < 0.05$). Conversely, statin supplementation also had no significant effects on the improvement of oxidative stress marker levels when compared with vinegar supplementation ($p > 0.05$). Furthermore, no significant differences were found in NOS levels across the different groups of the experimental mice ($p > 0.05$).

These results demonstrated that supplementation of hyperlipidaemic mice with sugarcane vinegar did not reduce oxidative damage in the liver of the experimental mice (except for the MD mice). Medium-dose supplementation significantly increased the liver SOD level ($p < 0.05$), whereas the low and high doses of vinegar supplementation showed no protective effect. Although high-fat diet

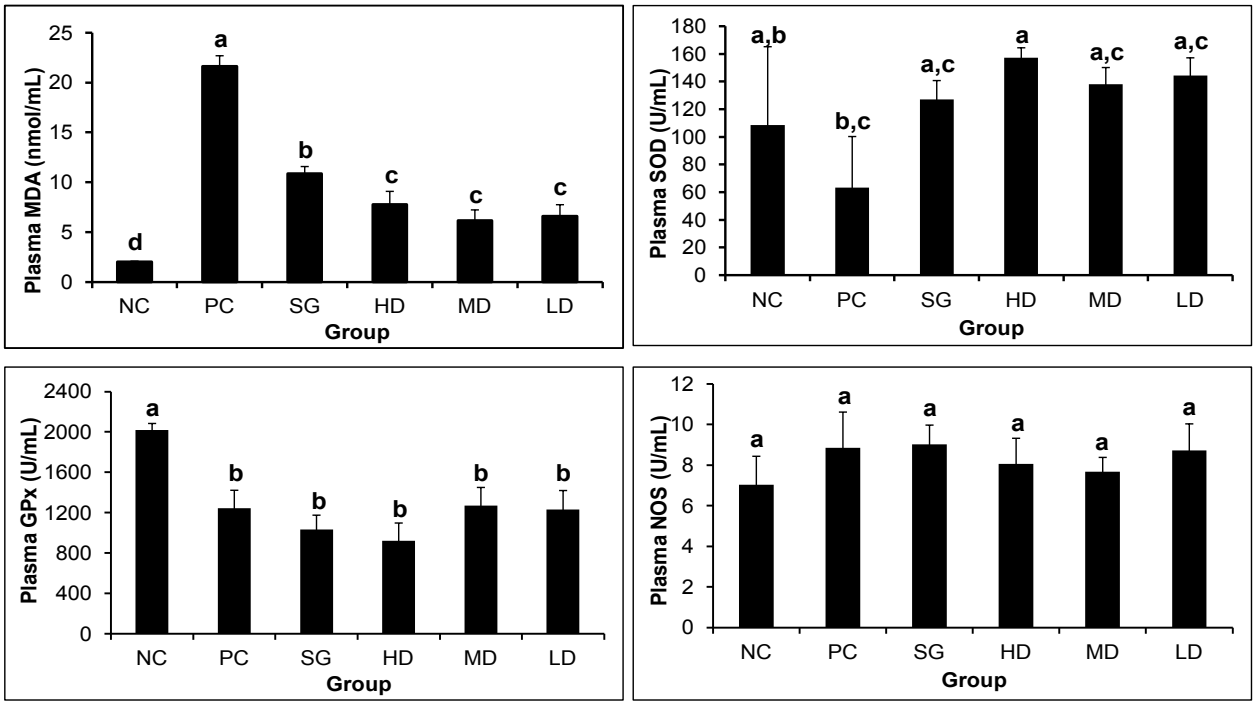

Figure 2. Effect of sugarcane vinegar supplementation on plasma oxidative stress markers of the experimental mice.

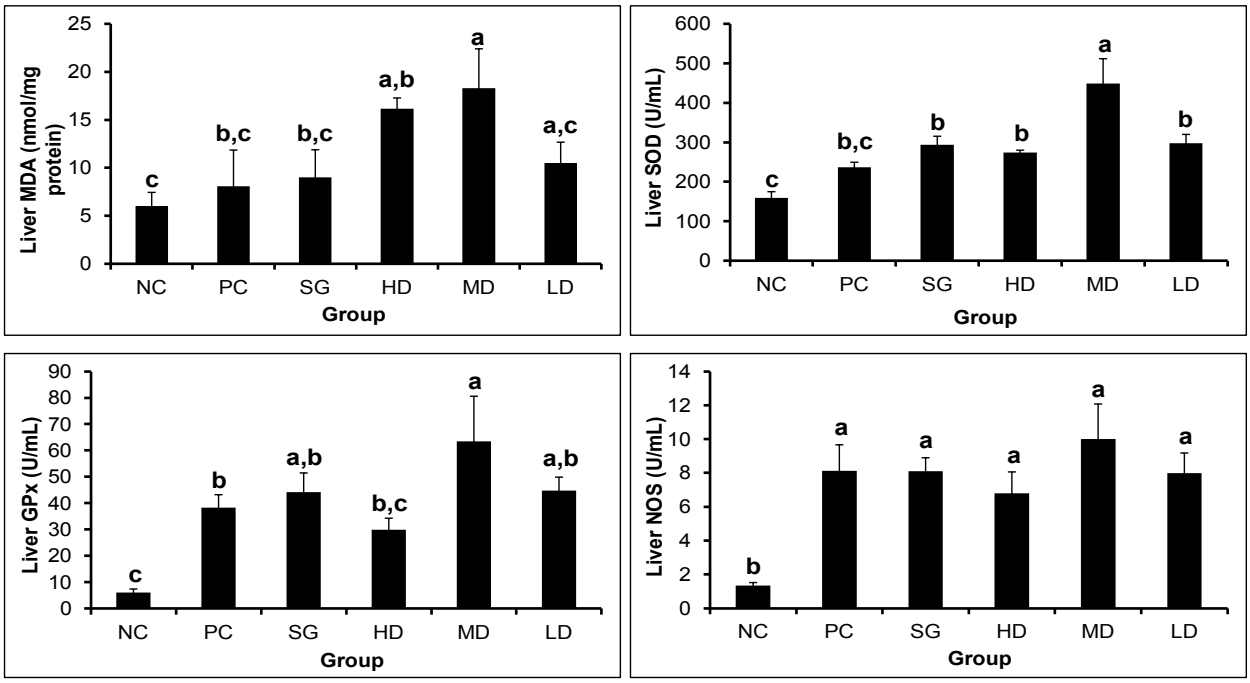

Figure 3. Effect of sugarcane vinegar supplementation on liver oxidative stress markers of the experimental mice.

did not cause significant increases in liver MDA and SOD levels in mice ($p > 0.05$), it significantly increased the liver GPx and NOS levels ($p < 0.05$). In addition, sugarcane vinegar supplementation at three different doses did not significantly reduce NOS in the liver ($p > 0.05$), and SG mice did not show significant amelioration of free radical-induced oxidative damage to the liver ($p > 0.05$).

Discussion

Obesity is a serious health problem that contributes to the high prevalence of metabolism-related disorders such as hyperlipidaemia, hypercholesterolaemia, hypertension, cardiovascular disorders, and non-alcoholic fatty liver disease (Jung and Choi, 2014). In many developing and developed nations, dietary intake patterns changed from low-carbohydrate and high-fibre diets to energy-dense diets, which contributed to the increasing prevalence of obesity (Hurt *et al.,* 2010).

The treatment of obesity is time-consuming as it is a complicated phenomenon with no simple solution. Although there are certain FDA-approved drugs without side effects, many obese individuals prefer dietary supplements for weight reduction to these drugs. This is because dietary supplements are typically rich in antioxidants and other essential nutrients, and exhibited benefits with respect to BW reduction and disease prevention (Alam *et al.,* 2014; Chao *et al.,* 2014); therefore, the weight reduction effect could be due to the functional amines present in the vinegar samples.

Literature has shown that vinegar (apple, balsamic, or sherry) contains high amounts (23.35 - 1445.2 μg/L) of biogenic amines (Ordóñez *et al.,* 2013). Amines such as histamine, agmatine, tyramine, putrescine, cadaverine, and spermidine are functional components that are used for weight loss via stimulating glucose intake by fat cells and lipolysis (Fontana *et al.,* 2000). Vinegar also contains bitter-sweet amino acids as major amino acids (Kong *et al.,* 2017), where the main functional group of the amino acids is an amine. Nevertheless, different types of vinegar have different biological activities (Yamashita, 2016).

MDA is an important marker of oxidative stress. Importantly, oxidative stress is considered to be an important indicator of health complications. MDA is the final product of lipid peroxidation of unsaturated fatty acids in the cell membrane that reacts with reactive oxygen species. It causes membrane protein and mitochondrial membrane lipid cross-linking, which generates a Schiff base, lowers the permeability of the membrane, affects the function of cells, and subsequently enhances the damage to the organisms. The level of MDA is an important indicator of lipid peroxidation in the biological system, and to some extent, it indirectly causes cell damage. A high-fat diet initiates lipid peroxidation in the liver tissue. This reaction is represented by the level of MDA in the tissues. Increased fat intake has also been reported to induce more nitric oxide (NO) generation; meanwhile, it

reduces the activity of antioxidative enzymes (SOD and catalase) and the concentration of glutathione (Poudyal *et al.,* 2010).

SOD is the only natural antioxidant enzyme that eliminates $O_2^{\bullet-}$ by converting it to H_2O_2, which is further catalysed to H_2O by GPx. The increase in the activities of these two enzymes denotes a higher antioxidative capability of an antioxidant in organisms. The vinegar-supplemented mice, as compared to the PC mice, had increased plasma SOD levels. This increase indicates that the oxidative damage in mice is suppressed by the functional components of the sugarcane vinegar. These functional components, including phenolic compounds and amino acids, play a protective role in the human cellular system via several mechanisms and antioxidant enzyme activities.

Similarly, GPx and these antioxidants protect the biological system of mice from oxidative damage. GPx is an important indicator for evaluating the protective effect of antioxidants against scavenging of reactive oxygen species. A high-fat diet could reduce the GPx activity in animals and humans. In addition, increased intake of high-fat and high-cholesterol food could reduce the ability of the liver to eliminate the free radicals from the body.

Excess NO generated from the organism produces a large number of free radicals, and so the rate-limiting enzyme of NO synthesis, NOS, was used to determine the status of NO. NOS is used to catalyse the reaction of generating NO from O_2 and L-arginine. The enzyme has been found to be widely distributed in blood vessel endothelium, nervous tissue, myocardium, respiratory tracts, and kidneys of both animals and humans. Increased intake of dietary fats induces production of more NO; excessive cellular NO might also induce a large number of free radicals and cause damage to the organs and cellular system.

The administration of vinegar-based beverages has been hypothesised to suppress NOS. However, the findings of the present work failed to support this hypothesis, given that the sugarcane vinegar supplementation showed no obvious effect in inhibiting NOS activity. In addition, there has been no previous report on the efficacy of sugarcane vinegar in suppressing NOS. Despite this, sugarcane vinegar has some protective effects against oxidative stress.

In the present work, a high-fat diet was used to induce hyperlipidaemia in the experimental mice. Besides inducing oxidative stress, the high-fat diet increased BW gain, organ mass, TG, TC, and blood sugar levels, and induced obesity. Previous studies have shown that a high-fat diet successfully up-regulates plasma LDL-c levels ($p < 0.01$), reduces plasma HDL-c levels, and increases glucose intolerance and insulin tolerance (Kaur, 2012; Auberval *et al.,* 2014).

The weight loss in PC mice, as evidenced in the findings of the present work, can be explained by the restricted caloric intake from carbohydrate as well as negative carbon balances of the high-fat diet-fed mice (Kekwick and Pawan, 1964). This weight loss could also be attributed to the up-regulation of ciliary neurotrophic factor (CNF) in the hypothalamus of the mouse (Severi *et al.,* 2013). In contrast, statin treatment down-regulated the expression of CNF. In addition to the absence of a weight-reducing effect, lovastatin improved oxidative stress in hyperlipidaemic mice (Chen *et al.,* 2005). As a result, the SG mice had slightly higher weight gain than the PC mice.

In addition to weight reduction and elevated HDL-c, the high-fat diet-fed mice in the present work had reduced plasma glucose levels. The negative carbon balance explains the increase in the use of glucose as calories by the mitochondria. The reduced plasma glucose level could also be due to the disruption of the uncoupling protein-2 gene, thus enhancing insulin secretion in the mice following the intake of a high-fat diet (Joseph *et al.,* 2002). Moreover, the reduction in plasma glucose level is also evidenced by the minor reductions in plasma α-amylase and lipase activities, especially in the sugarcane vinegar-supplemented mice. Furthermore, the results proved that supplementation with sugarcane vinegar further reduced body weight gain.

The results of the present work indicate that the intake of a high-fat diet induced fatty liver, which is evidenced by the significant increase in liver/BW index of the PC mice ($p < 0.05$). The literature supports our finding that high-fat diet-fed (42% fat in calories) mice had higher liver/BW index (%) than the low-fat diet control. Although there was a minor reduction in the liver/BW index of the sugarcane vinegar-supplemented mice, the non-significant changes in the levels of liver toxicity enzymes (Li *et al.,* 2020) indicate that sugarcane vinegar is safe for consumption even at a dose 20 times higher than that of the average oral daily intake by consumers for maintaining good health.

Vinegar is a functional beverage containing antioxidants (Dávalos *et al.,* 2005). Antioxidants such as phenolic compounds could effectively reduce blood sugar levels and insulin responses in human subjects (Ostman *et al.,* 2005), and improving hypertensive conditions (Murooka and Yamshita,

2008) and plasma lipid profiles (Fushimi *et al.,* 2006). Previous studies also reported that polyphenol-rich plant extracts could efficiently decrease blood glucose by inhibiting α-amylase and α-glucosidase activities as well as improving insulin tolerance of tissue (Shinde *et al.,* 2008; Sharma *et al.,* 2012).

He *et al.* (2017) found that sugarcane vinegar beverage contained 18.051 μg/mL of total phenolic content. As sugarcane juice contains a high amount of phenolic compounds, the vinegar developed from sugarcane juice could effectively improve glucose tolerance in animals and humans. Nevertheless, the data obtained in the present work revealed that sugarcane vinegar supplementation failed to improve glucose tolerance in the hyperlipidaemic mice. As α-amylase inhibitors are polysaccharide hydrolases (Yu *et al.,* 1999), they reduce the hydrolysis and digestion of carbohydrates in food and sugar, which reduces blood sugar levels and diminishes the symptoms related to hyperglycaemia and secretion of insulin, thus causing BW reduction due to the suppression of body fat accumulation (Fansheng, 2015). Based on the results obtained, we can conclude that sugarcane vinegar has a positive effect on weight reduction but not on the reduction of metabolic complications.

Conclusion

Sugarcane vinegar is beneficial to individuals who consume it daily as a supplemental beverage. It improves plasma lipid profile and lipid peroxidation markers in hyperlipidaemic mice, and contains phenolic compounds that act as strong antioxidants for reducing oxidative stress. A high dose of the sugarcane vinegar supplementation has a better protective effect than that of low and medium doses. Increased consumption of sugarcane vinegar would not cause any further reduction of oxidative stress, as the high concentration of phenolic compounds in the cellular system may burden the liver and other organs. The findings of the present work support the hypothesis that sugarcane vinegar is a good functional product for reducing body weight and oxidative stress. The present work employs the animal experimental method; future studies should use randomised human trials to test the research model.

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