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Anti-diabetes activity of Kombucha prepared from different snake fruit cultivars

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Abstract

Purpose – This paper aims to study the anti-diabetes activity of the Kombucha prepared from different snake fruit cultivars.

Design/methodology/approach – The juices of snake fruits of Suwaru, Madura, Pondoh and Bali cultivars were fermented for 14 days. Anti-diabetes activity of the products was analyzed. Twenty-four male albino Wistar rats were used and randomly divided into six experimental groups, i.e. four groups of the diabetic rats treated with the Kombucha, plus the normal group and diabetic control group. The Kombucha were orally administered to the streptozotocin induced-diabetic rats at 5 mL/kg body weight per day during the 28-day experiment. The fasting plasma glucose (FPG), oxidative stress indices (superoxide dismutase [SOD] activity and Malondialdehyde [MDA] level) and lipid profile of the blood plasma were measured. The pancreas was used for immunohistochemical study and β-cells quantification. Data were analysed by ANOVA followed by Fisher test using Minitab version 16.0.

Findings – FPG of the diabetic rats treated with the Kombucha (110.3-189.3 mg/dL) was significantly lower ($p = 0.000$) than the diabetic control group (413.3 mg/dL). Those were in line with the number of pancreatic β-cells of 42.1 in diabetic rats that lower ($p = 006$) than those in treated the diabetic rats (61.2-73.5). The treated diabetic rats had lower oxidative stress (SOD activity: 20.9-44.6 unit/100 μL with $p = 0.000$; MDA level: 0.37-0.48 ng/100 μL with $p = 0.000$) than those in the diabetic rats (SOD activity: 18.7 unit/100 μL; MDA level: 0.84 ng/100 μL). The treated diabetic rats also showed better lipid profile than those in the diabetic control rats. There were cultivar differences, and the Suwaru and Madura snake fruit Kombucha demonstrated the most potential for diabetes management.

Declaration of interest: The authors of this research declare no conflict of interest.

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Originality/value – This is the first study on in vivo anti-diabetes activity of snake fruit Kombucha prepared from different snake fruit cultivars.

Keywords In vivo, Anti-diabetes, Diabetic rats, Snake fruit, Kombucha

Paper type Research paper

Introduction

Diabetes mellitus is a chronic metabolic disease, in which homeostasis of the carbohydrate and lipid metabolisms are improperly regulated (Tiwari and Rao, 2002). Diabetes is a big health problem throughout the world, with more than 415 million currently diabetic and this is estimated to be about 642 million in 2040 (Ogurtsova *et al.*, 2017). Clinically, diabetic patients are characterized by high blood glucose level when the pancreas does not produce enough insulin or the insulin produced cannot be used effectively. Complications of diabetes are disabling and life threatening. Diabetes is also associated with fundamental changes in serum lipids profile (Govindji, 1990; Aloulou *et al.*, 2012; Rahimi-Madiseh *et al.*, 2017; Ani and Aginam, 2018).

Diabetes is mainly managed by insulin injections and administration of hypoglycemic drugs, which unfortunately can have several and severe adverse effects (Marín-Peñalver *et al.*, 2016). The search for an effective and safer treatment is, therefore, of great importance. Recently, functional foods and nutraceuticals are becoming prominent in diabetes therapeutic treatments (Metcalfe *et al.*, 2010; Ballali and Lanciai, 2012; Pereira *et al.*, 2016), and Kombucha tea has been reported as a therapeutic agent for hyperglycemia and dyslipidemia in diabetic animal models (Aloulou *et al.*, 2012; Srihari *et al.*, 2013). Kombucha tea is a beverage that has been consumed in Asia for over two millennia and is a notable traditional fermented foods globally (Jayabalan *et al.*, 2014). The putative health benefits associated with Kombucha tea have been largely attributed to its phenolic compounds. In addition, organic acids, vitamins, amino acids, antibiotics and a variety of micronutrients produce during the fermentation of Kombucha may also aid the health benefits to a reasonable extent (Vijayaraghavan *et al.*, 2000).

Several studies have demonstrated that Kombucha tea can be used in managing diabetes, and several mechanisms have been proposed to explain this desirable outcome (Bhattacharya *et al.*, 2011; Aloulou *et al.*, 2012; Srihari *et al.*, 2013). The proposed mechanisms include reduction in pancreatic β-cell damage, increase in insulin production, decrease uptake of glucose from the digestive system and increase in cellular glucose uptake (Bhattacharya *et al.*, 2011; Aloulou *et al.*, 2012; Srihari *et al.*, 2013). Tea is the main raw material for making Kombucha, whose desirable functional and nutraceutical properties have led to researches on other substrates for its manufacture (Gamboa-Gómez *et al.*, 2016; Lobanova *et al.*, 2016; Ayed *et al.*, 2017). Recently, Zubaidah *et al.* (2018) demonstrated the suitability of snake fruit (Salacca zalacca (Gaerth.) Voss) for making Kombucha. However, the potential of the snake fruit Kombucha in diabetes treatment has not been investigated. Therefore, the purpose of this research was to study the anti-diabetes activity of Kombucha prepared from different snake fruit cultivars.

Materials and methods

Materials

Snake fruits of commercial maturity were of cultivars Suwaru, Madura, Pondoh and Bali, and were obtained locally, so also commercial Kombucha starter and cane sugar. Streptozotocin (Sigma Aldrich, Germany), anthrone reagent (Merck 101468, Germany), sodium hydroxide (Merck 106462, Germany), Folin-Ciocalteau phenol reagent (Sigma F9252, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, Germany), thiobarbituric acid (Sigma Aldrich, Germany), lipid peroxidation malondialdehyde (MDA) assay kit (Sigma Aldrich, Germany), superoxide dismutase (SOD) assay kit (Sigma Aldrich, Germany), antibody anti-insulin (ScyTek Laboratories, Inc.) and the other chemicals used, were of analytical grade.

Snake fruit Kombucha preparation and analysis

As described before (Zubaidah *et al.*, 2018), the snake fruits were peeled, washed and cut into small sizes, before mixing (1:1, w/w) with water, juicing, filtering, sweetening (10 per cent, w/v) with the cane sugar, pasteurizing (65°C, 30 min), and cooling to ambient temperature prior to storing refrigerated in a sterile jar. The sweetened juices were aseptically inoculated (10 per cent, v/v) with the Kombucha starter and fermented (28 ± 3°C) for 14 days. The physicochemical and antioxidant properties of the products were analyzed as reported before (Zubaidah *et al.*, 2018).

Animal experiment

Male albino Wistar rats (age 2.5-3.0 months, body weight 150-200 g) were used. The experimental protocols and procedures of care and use of animals used in the present work were approved (ethical clearance No. KEP-749-UB) by the Ethics Committee. The rats were induced by an intra-peritoneally injection of freshly prepared streptozotocin (STZ, dissolved in 0.1 mol/L citrate buffer, pH 4.5) at a dose of 45 mg/kg body weight. Control rats were injected with the same volume of isotonic saline. After 72 hr., the plasma glucose was determined using a blood glucose test meter model AGM-2100 with strip glucotest Gluco Dr^{TM} No. 8 (Allmedicus, Korea). Before the plasma glucose was measured, the rats were fasted for 10-12 h, and the rats with a fasting plasma glucose (FPG) level greater than 250 mg/dL were classified as diabetic and used. There was 24 rats, which were randomly divided into six experimental groups, implying four replications per group. The six groups being the diabetic rats that received (5 mL/kg per day) the snake fruit Kombucha ([DM + KS Suwaru], [DM + KS Madura], [DM + KS Pondoh] and [DM + KS Bali]), plus the normal (Normal) and diabetic (DM) control rats. All the rats had access to a standard diet (Comfeed PARS; Japfa Comfeed Indonesia Tbk) and water was provided ad libitum. The snake fruit Kombucha were orally administered to the rats using an intra-gastric tube daily during the 28-day experiment. The initial and final FPG of the rats in various groups were measured. At the end of the experimental period, the rats were fasted overnight and sacrificed by dislocation cervical. Blood samples were collected from heart tissues and placed in a tube. Plasma was immediately separated by centrifugation (4°C, 1500g, 15 min, Hettig, Germany). The pancreas was dissected, washed and placed in 10 per cent neutral buffered formaline.

Biochemical analysis

The serum SOD activity and MDA level were measured using commercial kits. All assays were conducted according to the manufacturer instructions and protocols. Level of total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in serum were measured using the cholesterol oxidase-phenol/aminophenazone (CHOD-PAP) method, whereas the triglyceride (TG) level in the serum was measured using the glycerol-3-phosphate oxidase-phenol/aminophenazone (GPO-PAP) method (Jalali *et al.,* 2013).

Pancreas immunohistochemical study

Pancreas were fixed in 10 per cent neutral buffered formaline for 24 h and embedded in paraffin. Immunohistochemistry (IHC) staining was done according to Beesley (1995). After the deparaffinization and rehydration stage, the slides were incubated for 20 min with 0.5 per cent of H_2O_2 in methanol, then in DIVA solution. The slides were then washed with BPS, incubated with the primary antibody anti-insulin for 60 min, the secondary antibody (Universal Link) for 10 min, then trekavidin-HRP labeled for 10 min. After that, it was incubated for visualization by using diaminobenzidine (DAB) for 3 min and hematoxilin for 3 min. Pancreatic β-cells, which produced insulin would be shown as brown in color. Quantification of the cells was done (Suarsana *et al.*, 2010) by calculating the average of β-cells, which showed immune-reactivity to anti-insulin from five Langerhans islands at 400× of magnification.

Statistical analysis

Data are expressed as mean ± standard deviation for the four rats in each group (n = 4). The statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Fisher test (Stat View) using Minitab Ver.16.0. Statistical significance was accepted at $p < 0.05$.

Results

The characteristics of the snake fruit Kombucha are shown in Table I. There were no cultivar differences in the total sugar (p = 0.525) and total soluble solids (p = 0.088), but the other properties i.e. total acidity (p = 0.043), pH (p = 0.016) and total phenolic content (p = 0.000) were cultivar dependent. The *Suwaru* snake fruit Kombucha had the highest phenolic content (535.6 mg GAE/L). The differences in some of the parameters in Table I and those in Zubaidah *et al.* (2018) show location and batch differences, as those cultivars were obtained and processed at different times. This notwithstanding, the health benefits of the snake fruit Kombucha are undisputed in this study and our previous studies.

Anti-diabetes activity of the snake fruit Kombucha was indicated by the changes in the FPG levels before and after the treatments (Table II). At the 28th day, the FPG of the diabetic rats treated with the Kombucha (110.3-189.3 mg/dL) was significantly lower (p = 0.000) than the diabetic control group (413.3 mg/dL). It is noteworthy that the FPG levels of the diabetic rats treated with the *Madura* and *Suwaru* Kombucha were not significantly different (p = 0.000) from the normal control rats. The highest decrease in the FPG levels of the diabetic rats was 75.71 per cent when they were treated with the *Madura* Kombucha, and this is similar to those of the diabetic rats treated with the *Suwaru* Kombucha (75.66 per cent).

Table II also shows the SOD activity level of the diabetic control rats (18.7 unit/100 μL) was lower than the normal control rats (52.7 unit/100 μL). The STZ-induced diabetic rats treated with the snake fruit Kombucha had their SOD levels (20.9-44.6 unit/100 μL) significantly higher (p = 0.000) than those of the untreated diabetic rats. In line with the SOD results, the MDA level was significantly higher (p = 0.000) in the diabetic control rats (0.84 ng/100 μL) than in the normal control rats (0.28 ng/100 μL). Moreover, the MDA levels of

Snake fruit cultivar	Total acidity (%)	pH	Total sugar (%)	Total soluble solid (%)	Total phenolic content (mg GAE/L)
Suwaru	1.58 ± 0.14^{ab}	3.22 ± 0.09^{ab}	7.76 ± 0.03^{a}	12.9 ± 0.1^{a}	535.6 ± 2.0^{a}
Madura	1.64 ± 0.03^{a}	3.20 ± 0.12^{ab}	8.26 ± 0.17^{a}	12.9 ± 0.3^{a}	473.8 ± 8.6^{b}
Pondoh	1.71 ± 0.14^{a}	3.12 ± 0.02^{a}	8.28 ± 0.42^{a}	13.0 ± 0.1^{a}	377.1 ± 10.4^{c}
Bali	1.41 ± 0.14^{b}	3.28 ± 0.15^{b}	8.25 ± 0.87^{a}	13.9 ± 0.1^{a}	397.0 ± 16.8^{c}
p-value	0.043	0.016	0.525	0.088	0.000

Notes: *Values are means ± standard deviations (n = 3 for each group). Values in a column with the same letters are not significantly ($p > 0.05$) different. The statistical significance was evaluated by one-way ANOVA followed by Fisher test

Table I. Chemical characteristics of the snake fruit Kombucha* the diabetic rats treated with the snake fruit Kombucha (0.37-0.48 ng/100 μL) were significantly lower (p = 0.000) than those of the untreated diabetic rats. The *Suwaru* snake fruit Kombucha treatment resulted in the highest SOD level and the lowest MDA level of 44.6 unit/100 μL and 0.37 ng/100 μL, respectively.

Lipid profile including levels of LDL cholesterol, TC and TG in the diabetic control rats were significantly higher (p = 0.000) than those in the normal control rats. The levels of HDL cholesterol in the diabetic control rats were significantly lower (p = 000) than those in the normal control rats (Table III). Specifically, the *Suwaru*, *Madura* and *Bali* snake fruit Kombucha in the diabetic rats resulted in significantly lower levels of TG, TC and LDL cholesterol than those in the diabetic control rats, whereas the levels of HDL cholesterol were higher. It can be observed that the diabetic rats treated with the *Suwaru* snake fruit Kombucha had the best lipid profile, in which LDL cholesterol, TG and TC levels were not significantly different (p = 0.000) from those in the normal control rats. HDL cholesterol

Table II. Effect of the snake fruit Kombucha administration on the FPG level, number of pancreatic β-cells, SOD activity and the MDA serum levels in the rats*

Group	FPG level (mg/dL) 0 day	FPG level (mg/dL) 28th day	No. of pancreatic β-cells	SOD level (unit/100 μL)	MDA level (ng/100 μL)
Normal	119.5 ± 8.7^{b}	104.3 ± 3.4^{d}	114.9 ± 14.7^{a}	52.7 ± 1.8^{a}	0.28 ± 0.04^{e}
DM	463.8 ± 36.6^{a}	413.3 ± 8.3^{a}	42.1 ± 11.5^{c}	18.7 ± 1.4^{d}	0.84 ± 0.02^{a}
DM + KS Suwaru	453.0 ± 13.0^{a}	110.3 ± 2.9^{cd}	72.2 ± 17.9^{b}	44.6 ± 1.9^{b}	0.37 ± 0.03^{d}
DM + KS Madura	472.5 ± 42.4^{a}	114.8 ± 9.4^{cd}	73.5 ± 17.5^{b}	41.7 ± 0.8^{b}	0.48 ± 0.03^{b}
DM + KS Pondoh	466.0 ± 26.4^{a}	189.3 ± 15.4^{b}	63.8 ± 15.2^{bc}	30.8 ± 3.6^{c}	0.42 ± 0.04c
DM + KS Bali	445.0 ± 20.0^{a}	140.0 ± 14.4^{c}	61.2 ± 9.5^{bc}	20.9 ± 1.9^{d}	0.47 ± 0.02^{b}
p-value	0.000	0.000	0.006	0.000	0.000

Notes: *Values are means ± standard deviations (n = 4 for each group). Values of number of pancreatic β-cells, SOD and MDA levels obtained from the 28th day experiment. The animal experiments were designed as follows: normal rats, diabetic rats (DM) and diabetic rats with administration of snake fruit Kombucha (DM + KS) from different cultivar (Suwaru, Madura, Pondoh and Bali) at 5 mL/kg per day for 28 days. Values in a column with the same letters are not significantly ($p > 0.05$) different. The statistical significance was evaluated by one-way ANOVA followed by Fisher test

Table III. Effect of the snake fruit Kombucha administration on the lipid profile serum levels in the rats*

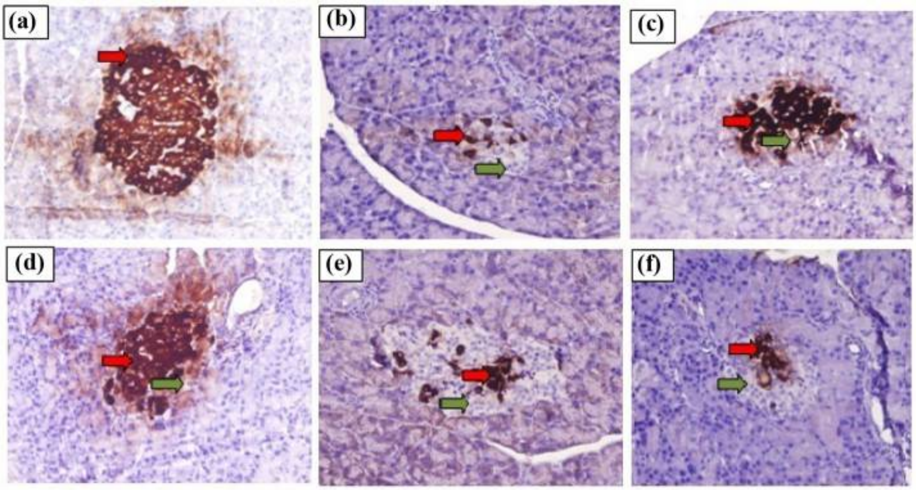
Group	HDL level (mg/dL)	LDL level (mg/dL)	TG level (mg/dL)	TC level (mg/dL)
Normal	59.3 ± 4.0^{a}	6.8 ± 1.0^{d}	48.0 ± 2.6^{d}	44.8 ± 2.6^{d}
DM	37.8 ± 5.7^{c}	14.3 ± 1.3^{a}	102.8 ± 6.9^{a}	75.3 ± 5.7^{a}
DM + KS Suwaru	46.8 ± 1.3^{b}	7.3 ± 1.0^{cd}	52.5 ± 6.8^{d}	49.0 ± 2.6^{cd}
DM + KS Madura	46.5 ± 2.1^{b}	10.3 ± 1.3^{bc}	74.5 ± 2.7^{c}	51.0 ± 3.5^{cd}
DM + KS Pondoh	43.8 ± 6.1^{bc}	12.8 ± 1.0^{ab}	97.8 ± 8.2^{ab}	66.8 ± 5.7^{b}
DM + KS Bali	44.0 ± 4.6^{bc}	10.3 ± 1.3^{bc}	92.0 ± 8.1^{b}	53.0 ± 4.2^{c}
p-value	0.000	0.000	0.000	0.000

Notes: *Values are means ± standard deviations (n = 4 for each group). Values of HDL, LDL, TG and TC levels obtained from the 28th day experiment. The animal experiments were designed as follows: normal rats, diabetic rats (DM), diabetic rats with administration of snake fruit Kombucha (DM + KS) from different cultivar (Suwaru, Madura, Pondoh and Bali) at 5 mL/kg per day for 28 days. Values in a column with the same letters are not significantly ($p > 0.05$) different. The statistical significance was evaluated by one-way ANOVA followed by Fisher test levels in the diabetic rats treated with the *Suwaru* and *Madura* snake fruit Kombucha were not significantly higher ($p = 0.000$) than the other snake fruit Kombucha treatments.

IHC analysis results are shown in Figure 1 and Table II. The size and shape of the Langerhans islands of the diabetic rats were smaller than those of the normal control rats, and that also had a very low immune reactive response (brown color) against anti-insulin to indicate a low level of insulin production. This is supported by the number of pancreatic β-cells that produced insulin in the diabetic rats, which was significantly lower ($p < 0.05$) than those in the normal control rats. Improvements of the Langerhans island structure and functions of the insulin secretion occurred in the diabetic rats treated with the snake fruit Kombucha compared to the diabetic control rats. Based on the data, the number of pancreatic β-cells that produced insulin in the diabetic rats treated with the snake fruit Kombucha was significantly higher ($p = 0.006$) than those in the diabetic control rats.

Discussion

Induction by using STZ caused destruction of β-cells of islets of Langerhans in the pancreas and led to lack of insulin secretion and increases in plasma glucose (Szkudelski, 2001; Srihari *et al.*, 2013). The findings of the present study showed that the rats treated with the snake fruit Kombucha from the Suwaru, Madura, Pondoh and Bali cultivars were significantly lower in SOD levels and higher in MDA levels that the normal control rats, and the reverse was the case when the snake fruit Kombucha were compared with the diabetic control rates (Table II). These beneficial effects of the snake fruit Kombucha on the measures of FPG could be due to the phenolics in the snake fruit Kombucha (Table I), such as flavonoids and tannins as previously reported (Zubaidah *et al.*, 2018). Although more data



Notes: (a) Normal rats; (b) diabetic rats; (c) diabetic rats with administration of Suwaru snake fruit Kombucha; (d) diabetic rats with administration of Madura snake fruit Kombucha; (e) diabetic rats with administration of Pondoh snake fruit Kombucha; (f) diabetic rats with administration of Bali snake fruit Kombucha. Red arrow: pancreatic β-cells which have immunoreactive to anti-insulin. Green arrow: endocrine cells which do not show immunoreactive to anti-insulin

Figure 1.
Effect of the snake fruit Kombucha administration on pancreatic cells in rats evaluated by IHC staining (400× magnification).

are required for any statistical relationships, from Tables I and II, a nominal positive SOP-phenolic content and negative MDA-phenolic content trends observed. Flavonoids and tannins act as antioxidants by donating hydrogen atoms from their hydroxyl aromatic groups (-OH) to bind free radicals, that may play a role in stimulating regeneration and protection of the architecture of pancreatic β-cells (Dipti *et al.*, 2003; Aloulou *et al.*, 2012; Zubaidah *et al.*, 2017). The IHC staining results (Figure 1 and Table II) indicated an improved performance of the pancreatic β-cells of the rats treated with the snake fruit Kombucha to stimulate insulin secretion. Babu *et al.* (2013) reported that pancreatic β-cells repair can increase insulin secretion and, furthermore, reduce blood glucose level. The blood glucose level can also decrease through the expression of glucose transporter 4 (GLUT-4). GLUT-4 is an active transporter of glucose from extra to intracellular in muscle and liver cells. Phenolic compounds can induce phosphorylation of insulin receptors to stimulate the activity of glucose transporters, one of which is GLUT-4 in cell membranes (Cao *et al.*, 2007; Nurrahma *et al.*, 2018). Increased expression of GLUT-4 can accelerate the transport of glucose into the cells to lower blood glucose levels. Similar to Kombucha tea, the ability of the snake fruit Kombucha in reducing the blood glucose level can be attributed to its ability in modulating immune system to decrease pancreatic β-cells damage. The snake fruit Kombucha was high in organic acid (Table I), and several organic acids have been identified in the Kombucha, including acetic acid, lactic acid and butyric acid (Zubaidah *et al.*, 2018). Phenolic compounds and organic acids in the Kombucha can reduce pancreatic α-amylase activity.

Chronic hyperglycemia can cause oxidative stresses, decrease activities of anti-oxidative systems and increase levels of reactive oxygen species (Dahech *et al.*, 2011). Oxidative environments can cause damage to cells through peroxidation of membrane lipids and glycosylation of proteins by free radicals (Rahimi-Madiseh *et al.*, 2017). Zubaidah *et al.* (2017) reported that STZ-induced diabetes can decrease SOD and increase MDA. In this study, STZ-induced diabetes decreased SOD and increased MDA levels in the serum (the normal and diabetic control rats), and upon treatments with the snake fruit Kombucha SOD increased and MDA decreased (the diabetic control and Kombucha-treated rats) in the serum of the STZ-induced diabetes rats (Table II). The mechanisms behind the SOD increase are still not clear, but several studies reported that it could be due to phenolic compounds increasing antioxidant enzymes. For example, Allium ampeloprasum extract increased catalase (Rahimi-Madiseh *et al.*, 2017); black garlic extract increased SOD and Glutathione Peroxidase (GSH-Px) (Wang and Sun, 2017); apple vinegar increased SOD (Nakamura *et al.*, 2010); and snake fruit vinegar increased SOD (Zubaidah *et al.*, 2017). Furthermore, as speculated above, SOD increases might be due to the presence of flavonoids (phenolic compounds). Ohkawa *et al.* (1979) revealed that flavonoids increased the activity of nuclear factorerytroid 2-related factor2 (Nrf2) that plays a role in synthesizing cellular antioxidants, including SOD.

This study also revealed that the injection of STZ also increased TG, TC and LDL cholesterol levels and decreased HDL cholesterol level in the rats (Table III). Insulin is an inhibitor of lipid mobilization from adipose tissues. Mobilization of fatty acids from triglyceride to the adipose tissues is helped by hormone-sensitive lipase, which can be activated by lack of insulin with a concomitant increase in serum lipid levels (Baradaran *et al.*, 2014). The reduction of insulin in diabetic conditions can also increase the activity of enzyme 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that contributes to cholesterol synthesis (Chogtu *et al.*, 2015; Abu-Hiamed, 2018). Treatments with the snake fruit Kombucha decreased LDL cholesterol, TC and TG levels of serum in the diabetic rats (Table III). Previous studies reported that treatments with Kombucha tea in diabetic rats reduced lipase activity (Aloulou *et al.*, 2012). Lipase is responsible for the hydrolysis of non-absorbable dietary triglycerides into absorbable monoglycerides and free fatty acids that can decrease serum TC and TG levels (Carriere *et al.*, 2001). Decreased levels of LDL cholesterol in the diabetic rats treated with the snake fruit Kombucha may be due to the presence of phenolic compounds (phenolic content-LDL cholesterol revealed a nominally negative trend), which can increase the expression of LDL receptors (LDLr) in the tissues. Increasing LDLr can lead to LDL cholesterol absorption in the blood, so that the level of LDL in the blood decreases (Morin *et al.*, 2008). Phenolic compounds can also inhibit the activity of HMG Co-A reductase in cholesterol synthesis, so that blood cholesterol levels do not increase (Ademosun *et al.*, 2015). Moreover, HDL cholesterol nominally shows a negative trend with TG levels as evident in Table III because when TG is transferred to the liver, the released proteins increase the formation of HDL cholesterol (Zubaidah *et al.*, 2014).

Acetic acid in the snake fruit Kombucha might also have a role in decreasing LDL cholesterol, TG and TC levels in the serum of the diabetic rats. That might be due to acetic acid inhibiting liver lipogenesis and activating protein kinase to maintain lipid homeostasis in the body (Yamashita *et al.*, 2007). In addition, acetic acid can inhibit metabolic pathway of cholesterologenesis (acetyl CoA to cholesterol) and lipogenesis (acetyl CoA into fatty acids and subsequently stored as triglycerides) in the liver, oxidation of fatty acids and stimulates fecal excretion of bile acids (Fushimi *et al.*, 2006).

Conclusion

Kombucha made from snake fruit cultivars Suwaru, Madura, Pondoh and Bali was effective as a therapeutic agent for anti-diabetes in streptozotocin-induced diabetic rats. The diabetic rats treated with the Suwaru snake fruit Kombucha showed a significantly decrease in blood glucose level; improved pancreas cells comparable to the normal control rats; improved oxidative stress status with low MDA and high SOD levels and improved lipid profile with low LDL cholesterol, TG and TC and high HDL cholesterol levels. The phenolic and organic acid contents of the Kombucha could explain its anti-diabetes activity, and position of snake fruit Kombucha as a functional beverage in the management of diabetes.

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