

In vivo evaluation of snake fruit Kombucha as hyperglycemia therapeutic agent

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***In vivo* evaluation of snake fruit Kombucha as hyperglycemia therapeutic agent**

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Abstract

This research was a part of development of functional beverage through fermentation of snake fruit juice with Kombucha consortium. The aim of this research was to study on *in vivo* evaluation of snake fruit Kombucha as hyperglycemia therapeutic agent. The snake fruit (*Salak Suwaru* cultivar) juice was fermented for 14 days with the Kombucha consortium. Streptozotocin induced diabetic rats were used in the *in vivo* evaluation. The snake fruit Kombucha was orally administered at different level for 28 days. The results revealed the treatment showed a significant fasting plasma glucose reduction in a range of 31-59%, consistent with improving of blood serum superoxide dismutase activity and malondialdehyde level. Immunohistochemical staining of pancreatic tissue proved a regeneration of the pancreatic beta cells in the groups of snake fruit Kombucha treatment compared to control group. Snake fruit Kombucha was proven as a hyperglycemia therapeutic agent in diabetic rats model.

Keywords

Snake fruit
Kombucha
In vivo evaluation
Hyperglycemia
Diabetic rats

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Introduction

Kombucha is a refreshing health-promoting beverage produced through fermentation by a symbiotic consortium of yeast species and acetic acid bacteria. In the Kombucha production, sugared tea infusion has been used as the fermentation substrate in traditional cultures. Researchers reported that several substrates other than tea had successfully applied in Kombucha production (Jayabalan *et al.*, 2014; Gamboa-Gomez *et al.*, 2016). In our previous study, we found that sugared snake fruit juices are good substrates for the fermentation with Kombucha consortium with desirable overall properties of the fermented beverage (Zubaidah *et al.*, 2018). Bioactive compounds including phenolic content, flavonoid, tannin and organic acids, along with the *in vitro* antioxidant and antibacteria activities have been detected in the snake fruit Kombucha. Therefore, the snake fruit Kombucha has a potential for development of functional beverages.

Many researchers reported antidiabetic

bioactivity of kombucha tea (Greenwalt *et al.*, 2000; Dufresne and Farnworth, 2000; Ernst, 2003; Aloulou *et al.*, 2012; Srihari *et al.*, 2013). Gamboa-Gomez *et al.* (2017) found that polyphenols contribute to the hypoglycemic effect of oak leaves Kombucha. Organic acids also contributed to the bioactivity (Fushimi *et al.*, 2005). It indicate that the snake fruit Kombucha has potential as antidiabetic bioactivity.

The aim of this research was to evaluate snake fruit Kombucha as a hyperglycemia therapeutic agent in diabetic animal models.

Materials and Methods

Materials

Snake fruit (*Salak Suwaru* cultivar) of commercial maturity were obtained from plantations in Malang, East Java, Indonesia. Commercial Kombucha starter was purchased from a local distributor, while cane sugar was bought from a local supermarket.

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Preparation of snake fruit juice and Kombucha

Preparation of the snake fruit juice and Kombucha were conducted as described in our previous research (Zubaidah et al., 2018). The sugared juice was inoculated with the Kombucha starter (1:10 w/w) and incubated for 14 days at room temperature.

Experimental design

Twenty five healthy 3 months old male Wistar rats were divided randomly into 5 groups with 5 replication. Group 1 (P0): normal rats; Group 2: diabetes mellitus/DM (P1); Group 3: DM with snake fruit Kombucha/KS at dose of 5 mL/kg BW/day (P2); Group 4: DM with KS at dose of 10 mL/kg BW/day (P3); and Group 5: DM with KS at dose of 15 mL/kg BW/day (P4). DM rats induced by STZ (Nacalai Tesque, Japan) intraperitoneally at a dose of 47.5 mg/kg body weight (BW). The rats were given access to standard diet and water ad libitum during 28 days experiment. The Group 3-5 were administered with snake fruit Kombucha orally once a day. FPG levels measurements were conducted on day 0 and day 28. At the end of the experiment, rats were sacrificed by cervical dislocation. Blood was used for the analysis of superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels, while pancreas was used for immunohistochemical (IHC) staining.

SOD activity assay

SOD activity assay was referred to Bannister and Calabrese (1987). Serum was obtained by centrifugation of blood of rats at 3,500 rpm for 10 mins. 200 µL of serum was put in the test tube, added with 200 µL of 100 mM EDTA, 100 µL of NBT, 100 µL of xanthinase, 100 µL of xanthine oxidase, then homogenized. The mixture was centrifuged at 3,000 rpm for 5 min. Supernatant was taken and added with distilled water to a 3 ml volume, then absorbance was measured at 580 nm. SOD activity was calculated by using standard curve.

Analysis of MDA

MDA analysis was referred to Rael et al. (2004). 200 µL of serum was put in the test tube, added with 500 µL of TCA 40% and homogenized. 200 µL of 1 N HCl, 500 µL of distilled water, 100 µL of 1% TBA were added, and then put in 100°C heater for 25 min. The mixture was cooled for 15 min and then centrifuged at 3,000 rpm for 10 min. Supernatant was removed and transferred into another tube. Distilled water was added to 3 ml, and absorbance was read at 532 nm. MDA level was calculated by using standard curve.

Immunohistochemical staining

After sacrificed, pancreas organ of rats was taken and fixed in buffered formalin 10% for 24 h. Furthermore, slides were made by standard methods using paraffin. IHC staining was referred to Beesley (1995). Visualization was used diaminobenzidine (DAB) for 3 min, while counterstain used Mayer's haematoxylin for 3 min. Insulin was visualized as brown color. Quantification was referred to Suarsana et al. (2010) by calculating the average of beta cells.

Statistical analysis

The data were analyzed by analysis of variance (ANOVA) and if any significant effect then further analyzed by LSD test at $p < 0.05$.

Results

Effect of snake fruit Kombucha on FPG levels

The changes in FPG levels before and after treatment are shown in Figure 1, significant reduction of FPG in a range of 31-59% occurred in the diabetic rats with snake fruit Kombucha treatment. This indicated the hyperglycemia therapeutic effect as a result of KS therapy.

SOD activity and MDA levels

Hyperglycemia can trigger enhancement of the production of free radicals that can exacerbate complications in DM patients (Bhattacharya et al., 2013; Sayyid and Fleshner, 2016). KS proved to increase the SOD activity and lower MDA levels significantly than DM group (Table 1). This demonstrates the ability of KS in reducing oxidative stress due to the condition of hyperglycemia in a diabetic rats models. KS capability in reducing oxidative stress in this study are consistent with other study on tea kombucha (Bhattacharya et al., 2013).

IHC staining and pancreatic beta cells regeneration

IHC staining analysis results are shown in Figure 2 and Figure 3. There was an improvement of langerhans islands structure and function of insulin secretion in the three-groups KS treatment (P2-P4) compared to DM group (P1) (Figure 2). The size and shape of the langerhans island of DM group were smaller and irregular than normal group (P0) and three-group KS treatment. In addition, the DM group showed a very low immunoreactive response (brown color) against the anti-insulin which indicated low levels of insulin production.

In the three KS treatment groups, the number and arrangement of endocrine cells look more homogeneous, and the intensity of the brown

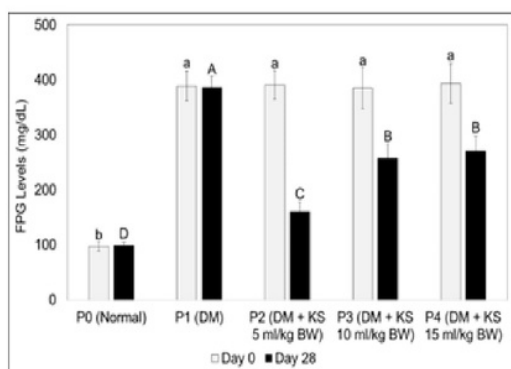


Figure 1. Changes in levels of FPG. Values are expressed as mean \pm SD. The same lowercase indicated no significant differences between the data on day 0, while the same uppercase showed no significant differences between the data on day 28 ($p < 0.05$). DM: Diabetes Mellitus, KS: *Salacca* var. *Suwaru Kombucha*, BW: Body Weight

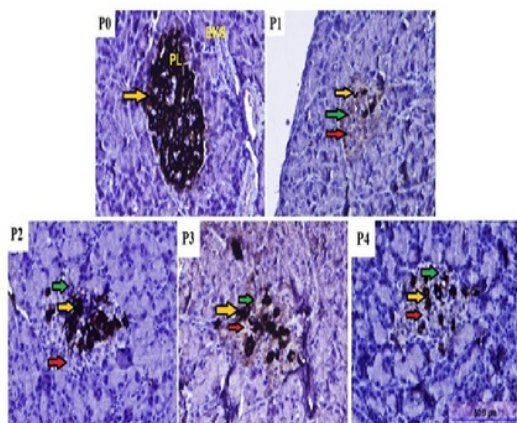


Figure 2. IHC staining at 400x magnification microscope. P0: Normal, P1: DM, P2: DM + KS 5 ml/kg BW/day, P3: DM + KS 10 ml/kg BW/day, P4: DM + KS 15 ml/kg BW/day, PL: Langerhans Island, EKS: Exocrine glands (acini), Yellow arrow: pancreatic beta cells which have immunoreactive to anti-insulin, Green arrow: endocrine cells which do not show immunoreactive to anti-insulin, Red arrow: empty space by necrosis, DM: Diabetes Mellitus, KS: *Salacca* var. *Suwaru Kombucha*, BW: Body Weight

color was increased compared to the DM group. This indicated the regeneration of beta cells in the three KS treatment groups. Those results indicated a hyperglycemia therapeutic effect of KS in the improvement of langerhans island structure and regenerations of pancreatic beta cells.

Discussion

The *in vivo* study demonstrated the KS ability as a hyperglycemia therapeutic agent. A decrease in

Table 1. SOD activity and MDA levels

Groups	SOD (unit/100 μ L)	MDA (ng/100 μ L)
P0 (Normal)	52.51 \pm 2.26 a	0.19 \pm 0.05 d
P1 (DM)	18.56 \pm 5.42 c	0.58 \pm 0.08 a
P2 (DM + KS 5 ml/kg BW)	41.95 \pm 6.21 b	0.45 \pm 0.04 b
P3 (DM + KS 10 ml/kg BW)	43.87 \pm 5.92 b	0.29 \pm 0.02 c
P4 (DM + KS 15 ml/kg BW)	46.75 \pm 2.78 ab	0.20 \pm 0.04 d

Values are expressed as mean \pm SD. The same letter show no significant differences between the data in the same column ($p < 0.05$). DM: Diabetes Mellitus, KS: *Salacca Suwaru Kombucha*, BW: Body Weight

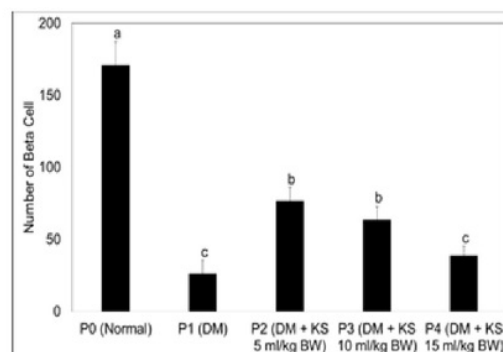


Figure 3. Average number of pancreatic beta cells. Values are expressed as mean \pm SD number of beta cells of five langerhans islands. The same letter indicated no significant difference between data ($p < 0.05$). DM: Diabetes Mellitus, KS: *Salacca* var *Suwaru Kombucha*, BW: Body Weight

blood glucose level in this study can be expected due to the mechanism of increasing production of insulin, decreasing uptake of glucose from the digestive system, and increasing cellular glucose uptake. The antioxidant activity in KS is thought to provide a protective effect and repairs the pancreatic beta cells so that it can improve insulin secretion. This is proved by the result of the IHC analysis (Figure 2 and Figure 3). Phenolic compounds are proven to increase insulin secretion from pancreatic beta cells (Johnson and de Mejia, 2016). Ultimately, improvement of insulin secretion will be able to lower blood glucose levels in hyperglycemia patients (Babu *et al.*, 2013). The therapeutic effect is thought to be the role of phenolic compounds and organic acids contained in KS. The fermentation process is able to significantly increase the content of phenolic compounds and organic acids in KS that increased antioxidant activity (Zubaidah *et al.*, 2018).

Hyperglycemia also can be treated by reducing the amount of glucose absorbed from the digestive system. Foodstuffs containing phenolic compounds

and organic acids are reported to decrease the absorption of glucose¹ from the digestive system (Ostman *et al.*, 2012; Aloulou *et al.*, 2012; Kallel *et al.*, 2012; Srihari *et al.*, 2013). Tea kombucha can provide inhibitory effects on the activity of alpha-amylase therefore¹ suppresses the increase in blood glucose levels (Aloulou *et al.*, 2012; Kallel *et al.*, 2012). Moreover, KS used in this study also contains organic acids and phenolic compounds (Zubaidah *et al.*, 2018). In addition, the dominant organic acid contained in KS is acetic acid. Acetic acid is reported to suppress the action of the disaccharidase and can slow gastric emptying time that have implications for the inhibition of glucose levels in blood (Ogawa *et al.*, 2000; Hlebowicz *et al.*, 2007). Acetic acid can increase blood glucose uptake by the liver and muscles to be converted into glycogen (Fushimi *et al.*, 2005).

In addition to lowering blood glucose levels, KS therapy can also prevent the negative effects of hyperglycemia conditions. Hyperglycemia can trigger enhancement of the production of free radicals that can exacerbate complications in DM patients (Bhattacharya *et al.*, 2013; Sayyid *et al.*, 2016). Exposure to free radicals can potentially cause increasing damage to biological macromolecules, especially lipids. KS has a high antioxidant activity (Zubaidah *et al.*, 2018) and proved to increase the SOD activity and lower MDA levels significantly than DM group (Table 1). Antioxidants in KS plays a role to improve the balance of oxidation status of the body, thereby reducing the workload of enzymatic antioxidants such as SOD, and reducing the formation of MDA. This demonstrates the ability of KS in reducing oxidative stress due to the condition of hyperglycemia in a diabetic rats models. KS capability in reducing oxidative stress in this study are consistent with other study on tea kombucha (Bhattacharya *et al.*, 2013).

Conclusions

The developed beverage snake fruit Kombucha at doses of 5-15 ml/kg body weight/day showed an ability as a hyperglycemia therapeutic agent in diabetic animal models. Further research on clinical evaluation of the snake fruit Kombucha will be conducted.

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Conflict of interest

The authors declare no conflict of interest

Ethical approval

Implementation research has approved by the Brawijaya University Research Ethics Committee (Animal care and use committee) with ethical clearance number of KEP-601-UB.

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