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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 administerred 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.

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 infussion 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 Bannisterb 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 xanthine, 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 a 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 sacrified, pancreas organ of rats was taken and fixed in buffered formalin10% for 24 h. Furthermore, slides were made by standard methods using paraffin. IHC staining was referred to Beesley (1995). Visualization was used diamino benzidine (DAB) for 3 min, while counterstaint used mayers haematoxilin 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% occured 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

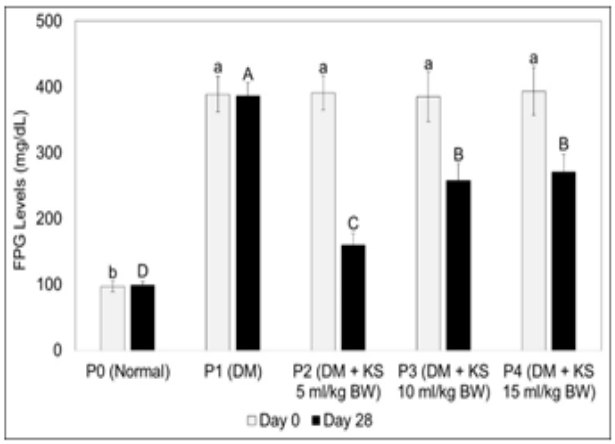

Figure 1. Changes in levels of FPG. Values are expressed as mean ± SD. The same lowercase indicated no significant differences between the data on day 0, while the same uppercase showed no significant differences between the data on day 28 ($p < 0.05$).DM: Diabetes Mellitus, KS: *Salacca* var. Suwaru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

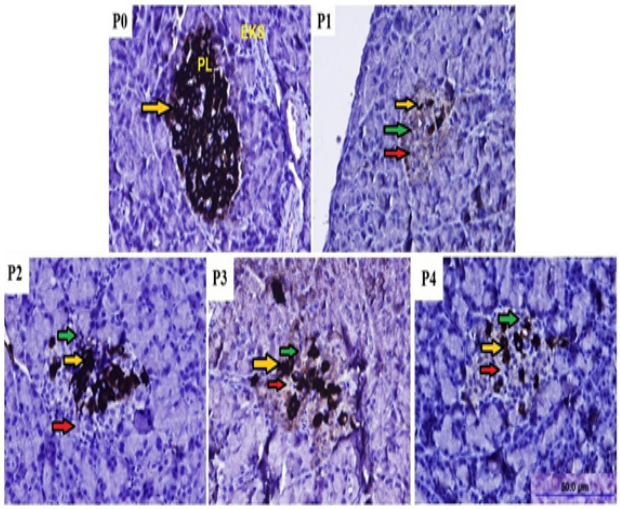

Figure 2. IHC staining at 400x magnification microscope. P0: Normal, P1: DM, P2: DM + KS 5 ml/kg BW/day, P3: DM + KS 10 ml/kg BW/day, P4: DM + KS 15 ml/kg BW/day, PL: Langerhans Island, EKS: Exocrine glands (acini), Yellow arrow: pancreatic beta cells which have immunoreactive to anti-insulin, Green 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

Tabel 1. SOD activity and MDA levels

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

Values are expressed as mean ± 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

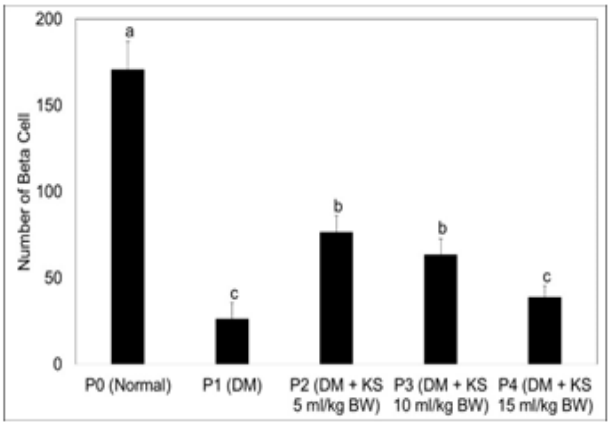

Figure 3. Average number of pancreatic beta cells. Values are expressed as mean ± SD number of beta cells of five langerhans islands. The same letter indicated no significant difference between data ($p < 0.05$).DM: Diabetes Mellitus, KS: *Salacca* var Suwaru Kombucha, BW: Body Weight

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 hiperglicemia 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 glucose 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 therefore 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 glicogen (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.

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.

References

Aloulou, A., Hamden, K., Elloumi, D., Ali, M.B., Hargafi, K., Jaouadi, B., Ayadi, F.,Elfeki, A. and Ammar, E. 2012. Hypoglycemic and antilipidemic properties of Kombucha tea in alloxan-induced diabetic rats. BMC Complement. Alternative Medicine 12 63–71.

Babu, P.V.A., Liu, D. and Gilbert, E.R. 2013. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. Journal of Nutrition and Biochemistry 24(11): 1777–1789.

Bannisterb, J.V. and Calabrese, L. 1987. Assays for Superoxide Dismutase. Methods of Biochemistry Analysis 32: 279-312.

Beesley, J.E. 1995. Immuno-cytochemistry: A Practical Approach. New York: Oxford University Press.

Bhattacharya, S., Gachhui, R., and Sil, P.C. 2013. Effect of Kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. Food Chemistry and Toxicology 60: 328-340.

Dufresne, C. and Farnworth, E. 2000. Tea, kombucha, and health: a review. Food Research International 33: 409–21.

Ernst, E. 2003. Kombucha: a systematic review of the clinical evidence. Forsch Komplementarmed Klass Naturheilkd 10: 85–87.

Fushimi, T. and Sato, Y. 2005. Effect of acetic acid feeding on the circadian changes in glycogen and metabolites of glucose and lipid in liver and skeletal muscle of rats. Brazilian Journal of Nutrition 94: 714–719.

Gamboa-Gómez, C.I., González-Laredo, R.F., Gallegos-Infante, J.A., Pérez, M.M.L.,Moreno-Jiménez, M.R., Flores-Rueda, A.G. and Rocha-Guzmán, N.E. 2016. Antioxidant and angiotensin-converting enzyme inhibitory activity of Eucalyptus camaldulensis and Litsea glaucescens infusions fermented with Kombucha consortium. Food Technology and Biotechnology 54(3): 367–374.

Gamboa-Gómez, C.I., Simental-Mendia, L.E., González-Laredo, R.F., Alcantar-Orozco, E.J., Monserrat-Juarez, V.H., Ramirez-Espana, J.C., Gallegos-Infante, J.A., Moreno-Jiménez, M.R. and Rocha-Guzmán, N.E. 2017. In vitro and in vivo assessment of anti-hyperglycemic and antioxidant of Oak leaves (*Quercus convallata* and *Quercus arizonica*) infusions and fermented beverages. Food Research International 102: 690–699.

Greenwalt, C.J., Steinkraus, K.H. and Ledford, R.A. 2000. Kombucha, the fermented tea: microbiology, composition, and claimed health effects. Journal of Food Protection 63(7): 976-981.

Hlebowicz, J., Darwiche, G., Björgell, O. and Almer, L.O. 2007. Effect of apple cider vinegar on delayed gastric emptying in patients with type 1 diabetes mellitus: a pilot study. BMC Gastroenterology 7: 46.

Jayabalan, R., Malbaˇsa, R.V., Lonˇcar, E.S., Vitas, J.S. and Sathishkumar, M. 2014. A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea tungus. Comprehensive Reviews in Food Science and Food Safety 13: 538-550.

Johnson, M.H. and de Mejia, E.G. 2016. Phenolic Compounds from Fermented Berry Beverages Modulated Gene and Protein Expression To Increase Insulin Secretion from Pancreatic β-Cells *in Vitro*. Journal of Agriculture and Food Chemistry 64(12): 2569–2581.

Kallel, L., Desseaux, V., Hamdi, M., Stocker, P. and Ajandouz, E.H. 2012. Insights into the fermentation biochemistry of Kombucha teas and potential impacts of Kombucha drinking on starch digestion. Food Research International 49: 226–232.

Liu, X., Kim, J.K., Li, Y., Li, J., Liu, F. and Chen, X. 2005. Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. Journal of Nutrition 135: 165–171.

Ogawa, N., Satsu, H., Watanabe, H., Fukaya, M., Tsukamoto, Y., Miyamoto, Y. and Shimizu, M. 2000. Acetic Acid Suppresses the Increase in Disaccharidase Activity That Occurs during Culture of Caco-2 Cells. Journal of Nutrition 130(3): 507–513.

Ostman, E., Granfeldt, Y., Persson, L. and Bjorck, I. 2005. Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. European Journal of Clinical Nutrition 59(9): 983–988.

Rael, L.T., Thomas, G.W., Craun, M.L., Curtis, C.G., Bar-Or, R. and Bar-Or, D. 2004. Lipid peroxidation and the thiobarbituric acid assay: standardization of the assay when using saturated and unsaturated fatty acids. Journal of Biochemistry and Molecular Biology 37(6): 749-752.

Sayyid, R.K. and Fleshner, N.E. 2016. Diabetes mellitus type 2: a driving force for urological complications. Trends in Endocrinology and Metabolism 27(5): 249-261.

Srihari, T., Karthikesan, K., Ashokkumar, N., and Satyanarayana, U. 2013. Antihyperglycaemic efficacy of kombucha in streptozotocin-induced rats. Journal of Functional Foods 5(4): 1794-1802.

Suarsana, I.N., Priosoeryanto, B.P., Bintang, M. and Wresdiyati, T. 2010. Profile of blood glucose and ultrastucture of beta cells pancreatic islet in alloxan compound induced rats. Indonesian Journal of Animal and Veterinary Sciences. 15(2): 118-123.

Szkudelski, T. 2001. The mechanism of alloxan and streptozotocin action of b-cells of the rat pancreas. Physiology Research 50: 537–546.

Zubaidah, E., Dewantari, F.J., Novitasari, F.R., Srianta, I. and Blanc, P.J. 2018. Potential of snake fruit (*Salacca zalacca* (Gaerth.) Voss) for the development of a beverage through fermentation with the Kombucha consortium. Biocatalysis and Agricultural Biotechnology 13: 198-203.