

In vivo evaluation of faloak (*Sterculia quadrifida* R.Br) stem bark kombucha as hyperglycemia and therapeutic agent

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Abstract. This study aims to investigate the potential differences in the anti-hyperglycemia and antioxidant effects of fermented faloak (*Sterculia quadrifida* R.Br) stem bark (FSB) kombucha and FSB brew (without fermentation) in alloxan-induced diabetic rats. Black tea (BT) kombucha was used as a kombucha control. FSB kombucha, BT kombucha, and FSB brew were administered orally at a dose of 5 mL/Kg bw/day into the alloxan-induced diabetic rats for 28 days. Fasting blood glucose (FBG), body weight, superoxide dismutase activity, malondialdehyde levels, and pancreatic histopathology of the rats were analyzed. The results of this study showed that FSB kombucha, BT kombucha, and FSB brew were able to effectively reduce FBG, increase superoxide dismutase (SOD) activity, reduce malondialdehyde (MDA) levels, improve lipid profile, and repair pancreatic β -cells in the islets of Langerhans. The administration of FSB kombucha significantly ($P < 0.05$) showed a more optimal potency than the unfermented FSB brew, while the ability was comparable to that of BT kombucha. Thus, faloak (*Sterculia quadrifida* R.Br) stem bark can be used as an alternative substrate other than black tea in the making of kombucha.

Keywords: *Sterculia quadrifida* R.Br; stem bark; kombucha; black tea; anti-diabetic; dyslipidemia

1 Introduction

Sterculia quadrifida R.Br is a local herbal plant spread across almost the entire island of Timor, East Nusa Tenggara Province, Indonesia. The name 'faloak' is a local name extensively used by the local community, where faloak stem bark (FSB) is traditionally boiled, brewed, and consumed to treat various diseases. According to Lulan et al. [1] there is currently not much scientific evidence that leads to the efficacy of the stem bark of faloak (*Sterculia quadrifida* R.Br), especially in the functional food sector. Lulan et al. [1] reported the presence of flavonoid and phenol compounds in FSB, thus making it potential as an antiviral [2], immunomodulator [3, 4], and anticancer [5].

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To increase the efficacy and attractive flavor of FSB drinks, FSB is used as a substrate for kombucha fermentation, considering that currently many researchers have explored the use of other substrates to make kombucha because of their various benefits for our health [6]. It has been reported by several researchers that tea kombucha has the potential as anticancer, antimicrobial [7], anti-hypercholesterolemic [8], and anti-diabetic agents [9–11]. In addition, Sknepnek et al. [12] found that kombucha has the potential to increase immune response. The efficacy of kombucha drinks is supported by the high antioxidant activity and the production of polyphenols and organic acids during fermentation [13]. Several studies proved that there is an increase in the bioactive compounds of black tea after being fermented into kombucha [9, 14]. Bhattacharya et al. [14] and Banerjee et al. [15] also reported an increase ability of fermented black tea in in-vivo treatment compared to unfermented one. In this study, the anti-diabetic ability of fermented FSB drink was investigated for the first time by administering the kombucha consortium as well as unfermented FSB brew in alloxan-induced diabetic rats.

The number of people with diabetes is increasing every year, thus prompting the need for special attention to this matter. Saedi et al. [16] estimated that the prevalence of diabetes will increase by 10.9% (approximately 700 million) globally in 2045. In addition, the condition is exacerbated by the alleged connection with covid-19 [17]. Aloulou et al. [9] explained in their research that black tea kombucha was able to lower blood glucose, suppress the activity of α -amylase and lipase enzymes, and improve the histological structure of pancreas of alloxan-induced diabetic rats. Gamboa-Gómez et al. [18] reported that the administration of oak leaf kombucha was able to reduce fasting blood glucose levels and glucose triglyceride index (TyG) by 52-66% and 5.9-7.5% respectively. In addition, it was able to inhibit the activity of α -amylase and α -glucosidase enzymes. A research conducted by Zubaidah et al. [10] also proved that the administration of snake fruit kombucha for 28 days could reduce blood glucose and malondialdehyde (MDA) levels, increase the activity of the superoxide dismutase (SOD) enzyme, and increase the productivity of pancreatic β -cells in diabetes-induced rats.

The high potential of kombucha as an anti-diabetic agent has encouraged the implementation of this study, considering that there are currently no studies on the use of faloak (*Sterculia quadrifida* R.Br) stem bark as an alternative substrate for kombucha. Therefore, the objective of this study is to investigate the differences in antioxidant and anti-diabetic potencies of FSB kombucha and FSB brew in alloxan-induced diabetes mellitus rats.

2 Materials and methods

2.1 Materials

The faloak stem bark (FSB) used in this study was taken from Kupang City, East Nusa Tenggara, Indonesia. Black tea was obtained from a commercial outlet in Malang, East Java, Indonesia, with the brand Teh Tong Tji (PT. Cahaya Tirta Rasa). The kombucha culture was obtained from an Indonesian commercial online outlet, namely IndoKombucha, located in Bandung, West Java. Alloxan-monohydrate (Sigma-Aldrich, Germany), folin ciocalteu (Merck, Germany), anthrone (Sigma-Aldrich, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany), thiobarbituric acid (TBA) (Merck, Germany), etilendiamintetraasetat (EDTA) (Merck, Germany), nitroblue tetrazolium (NBT) (Merck, Germany).

2.2 Preparation of faloak stem bark (FSB) and black tea (BT)

The procedure for making FSB tea was adopted from Rollando et al. [19]. The FSB that had been taken from the trees were cleaned and air-dried at room temperature for 4 days, then crushed and sieved with a size of 40-60 mesh. FSB powder was analyzed for moisture content to obtain a moisture content of <10%. Every 2 grams of FSB powder was then added to each tea bag. The Black tea (BT) used in this study was obtained from a commercial outlet.

2.3 Preparation and analysis of FSB kombucha and BT kombucha

The preparation of FSB kombucha and BT kombucha was adapted to the modified Sreeramulu et al. [20] method. 500 ml of water was boiled and then 10% (w/v) sugar was added. After that, 8g of FSB (the best concentration tested previously) and 2g of BT were added and boiled separately for 10 minutes. The decoctions of FSB and BT were put in sterile glass jars, then tightly closed and cooled to 25°C. Both were then added with 10% (v/v) liquid culture, covered with a sterile cloth, and stored at room temperature (25°C) for 14 days. Sampling was repeated 3 times. Each sample and replication was analyzed for chemical and bioactive properties on days 0 and 14 [21].

2.4 Animal experiment and analysis

The animal testing conducted in this study referred to a research by Zubaidah et al. [10] where 25 males white wistar rats aged 2.5 months were divided into 5 treatment groups, each consisting of 5 rats. Group 1 (P0) consisted of normal rats; Group 2 (P1) contained DM rats; Group 3 (P2) was for DM rats given 5 ml/kg body weight (BW) of BT kombucha for 28 days; Group 4 (P3) comprised of DM rats given 5 ml/kg BW of FSB kombucha for 28 days; and Group 5 (P4) included DM rats given 5 ml/kg BW of FSB brew for 28 days. The DM rat model was carried out by inducing alloxan monohydrate (ALX) at a dose of 150 mg/kg body weight [9]. They were fed standard Comfeed PARS by Japfa Comfeed Indonesia Tbk. and given *ad libitum* water.

The fasting blood glucose levels of the rats were analyzed on days 0 and 28 using a blood glucose test meter (GlucoDr AGM-2100). In the last treatment (Day 28), all rats were dislocated and their blood was taken from the heart to analyze the activity of superoxide dismutase (SOD), malondialdehyde (MDA), and the levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL). Then, the pancreas was taken for histopathological analysis.

2.5 Pancreatic immunohistochemical studies

The immunohistochemical study was based on the method described by Zubaidah et al. [10]. The pancreas of the rats was taken during surgery and fixed in 10% buffered formalin for 24 hours. Then, the paraffin slides were cut. Slide preparations were stained with immunohistochemistry with the use of insulin antibodies. The preparations were observed through a light microscope (Olympus BX51) with 400x magnification, with the red color displayed on the object as a result of visualization of the insulin from pancreatic β -cells.

2.6 Statistical analysis

The data were analyzed by applying the analysis of variance (ANOVA). When showing a difference, it was further tested with the LSD test at a significant level of 0.05.

3 Results and discussion

3.1 Results

3.1.1. Chemical characteristics of FSB kombucha and BT kombucha

The brewing of FSB and control black tea which was fermented using kombucha culture for 14 days had an impact on changes in total acid, pH, and total sugar. There was a decrease in pH and total sugar as well as an increase in total acid in both samples during fermentation, thus indicating a significant difference between Day 0 and Day 14 of fermentation (Table 1). Both kombucha samples did not have a significant effect on the values of pH and total acid, but they did on the total sugar. With these changes, it is assumed that there is a role for microorganisms during the fermentation process.

In this study, the fermentation process also resulted in the changes in the bioactive compounds and the antioxidant activities produced in FSB kombucha or BT kombucha, as shown in Table 1. There was a significant difference between the duration of fermentation on Day 14 and Day 0. The type of kombucha used also showed significant differences in the bioactive contents during fermentation (Table 1). The antioxidant activities between the two types of kombucha showed a significant difference at the end of fermentation; FSB kombucha showed its activity of 82.21% in counteracting DPPH free radicals.

Table 1. Chemical characteristics of FSB kombucha and BT kombucha.

Parameters	Type of kombucha			
	FSB kombucha		BT kombucha	
	Day 0	Day 14	Day 0	Day 14
pH	3.86 ± 0.02	2.78 ± 0.48***a	4.03 ± 0.10	3.04 ± 0.05***a
Total acid (%)	0.19 ± 0.03	0.62 ± 0.12***a	0.19 ± 0.02	0.67 ± 0.09***a
Total sugar (%)	9.09 ± 0.24	7.15 ± 0.13***a	9.21 ± 0.17	7.62 ± 0.14***b
Total phenols (mg/L GAE)	240.21 ± 8.85	467.92 ± 8.37***b	262.67 ± 13.56	475.13 ± 3.82***a
Total flavonoids (mg/L QE)	3497.00 ± 38.44	4134.78 ± 97.54***a	3369.22 ± 63.36	3981.44 ± 26.94***b
DPPH antioxidant activities (%)	70.79 ± 0.56	82.21 ± 0.32***a	70.41 ± 0.85	80.15 ± 0.32***b

3.1.2. Fasting blood glucose (FBG) of the experimental rats

Blood glucose levels of the rats were measured on days 0 and 28; the measurement data are shown in Figure 1. FBG measurement data on Day 0 showed no significant difference ($p > 0.05$) between the group of DM rats (P1) and the groups of P2, P3, and P4 rats, while showing a significant difference ($p < 0.05$) with the group of normal rats (P0). On the 28th

day, there was a decrease in FBG in the P2, P3, and P4 groups, thus indicating a significant difference among the treatment groups. The groups of DM rats given FSB kombucha (P3) and BT kombucha (P2) showed lower FBG levels than the group of those given FSB brew (P4). FSB kombucha showed a decrease from 331.6 mg/dL (Day 0) to 120.8 mg/dL (Day 28), while FSB brew from 369.2 mg/dL to 197.4 mg/dL.

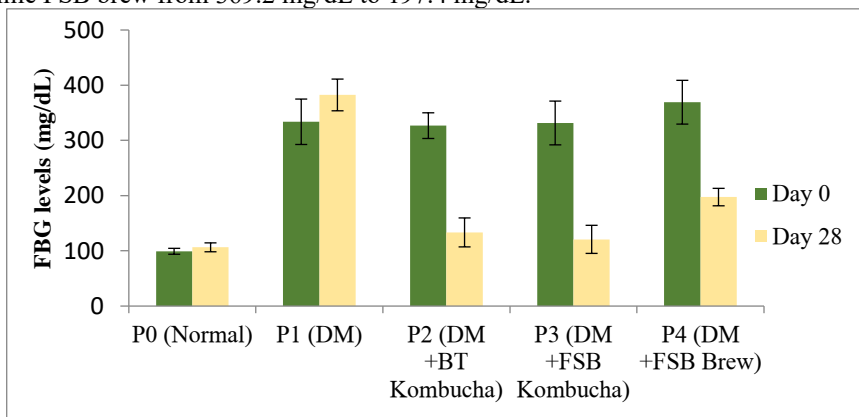


Fig. 1. The effect of BT kombucha, FSB kombucha, and FSB brew administrations on FBG levels of experimental rats for 28 days.

3.1.3. Malondialdehyde (MDA) and superoxide dismutase (SOD) levels

The measurement results of MDA and SOD levels are shown in Table 2. The group of alloxan-induced DM rats showed an increase in MDA levels and a decrease in SOD activity. These changes were observed at the end of the treatment (Day 28), thus showing a significant difference ($p < 0.05$) with the control group of normal rats. The opposite outcome was seen in DM rats given BT kombucha, FSB kombucha, and FSB brew which showed a downward trend in MDA levels accompanied by an increase in SOD activity at the end of the treatment.

Table 2. The effect of BT kombucha, FSB kombucha, and FSB brew administrations on MDA levels and SOD activity of experimental rats.

Treatment	MDA level (ng/ml)	SOD level (U/ml)
P0 (Normal)	316.00 ± 9.92 ^d	48.89 ± 1.53 ^a
P1 (DM)	436.44 ± 25.73 ^a	25.44 ± 7.02 ^d
P2 (DM +BT kombucha)	342.67 ± 26.00 ^c	44.76 ± 1.50 ^{ab}
P3 (DM +FSB kombucha)	334.00 ± 14.54 ^{cd}	43.96 ± 2.32 ^b
P4 (DM +FSB brew)	384.00 ± 4.20 ^b	38.93 ± 1.38 ^c

3.1.4. Lipid profile

Table 3 shows the results of lipid profiles measurements which include the levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) in the serum of DM rats during the 28 days of treatment. The DM rats (P1) group showed increased levels of TC, TG, and LDL, and a decreased HDL level compared to the normal rats (P0) group. Meanwhile, in the groups of DM rats given BT kombucha (P2), FSB kombucha (P3), and FSB brew (P4), a decrease in TC, TG, and LDL levels accompanied by an increase in HDL level were shown. The administration of FSB kombucha and BT kombucha showed the ability to improve lipid profile levels which was

more effective than FSB brew. The P2 and P3 groups showed no significant difference with the normal rats (P0) group.

Table 3. The effect of BT Kombucha, FSB Kombucha, and FSB Brew administrations on serum lipid profile levels in experimental rats.

Treatment	TC (mg/ml)		TG (mg/ml)		HDL (mg/ml)		LDL (mg/ml)	
P0 (Normal)	50.6	± 8.17 ^b	51.8	± 19.64 ^c	53.2	± 7.46 ^a	33	± 8.31 ^c
P1 (DM)	71.8	± 8.04 ^a	163	± 30.55 ^a	30.4	± 4.10 ^c	63.2	± 10.43 ^a
P2 (DM +BT Kombucha)	53.4	± 5.41 ^b	53.2	± 16.12 ^c	41.6	± 8.85 ^b	44.4	± 7.86 ^{bc}
P3 (DM +FSB Kombucha)	51.2	± 4.21 ^b	52.4	± 15.01 ^c	46.8	± 9.52 ^a	34.4	± 8.79 ^c
P4 (DM +FSB Brew)	56.8	± 7.05 ^b	85.6	± 32.36 ^b	45.2	± 7.85 ^a	49.6	± 10.16 ^b

3.1.5. Pancreatic immunohistochemical studies

Figure 2 displays the histopathological observations of the islets of Langerhans through immunohistochemical (IHK) staining while Table 5 shows the number of pancreatic β -cells of the experimental rats. There were differences in the shape, size, and number of cells between DM control rats and normal rats. DM control rats (P1) were also observed to have differences from the DM rats given FSB kombucha (P3), BT kombucha (P2), and FSB brew (P4). The diversity in color intensity (brown color) among the groups as an indicator of a reactive immune response to anti-insulin shows differences in the level of insulin produced (Fig. 1). In Table 5, the number of pancreatic β -cells that are active in insulin production in DM rats showed a significant difference ($p < 0.05$) from the normal rats. In the groups of DM rats given FSB kombucha, BT kombucha, and FSB brew, there were an increase in the number of cells as well as changes in structure, shape, and color intensity that led to the improvement of the islets of Langerhans. However, the rats treated with FSB kombucha showed a more effective change and a significant increase in the number of β -cells ($p < 0.05$) compared to those given FSB brew.

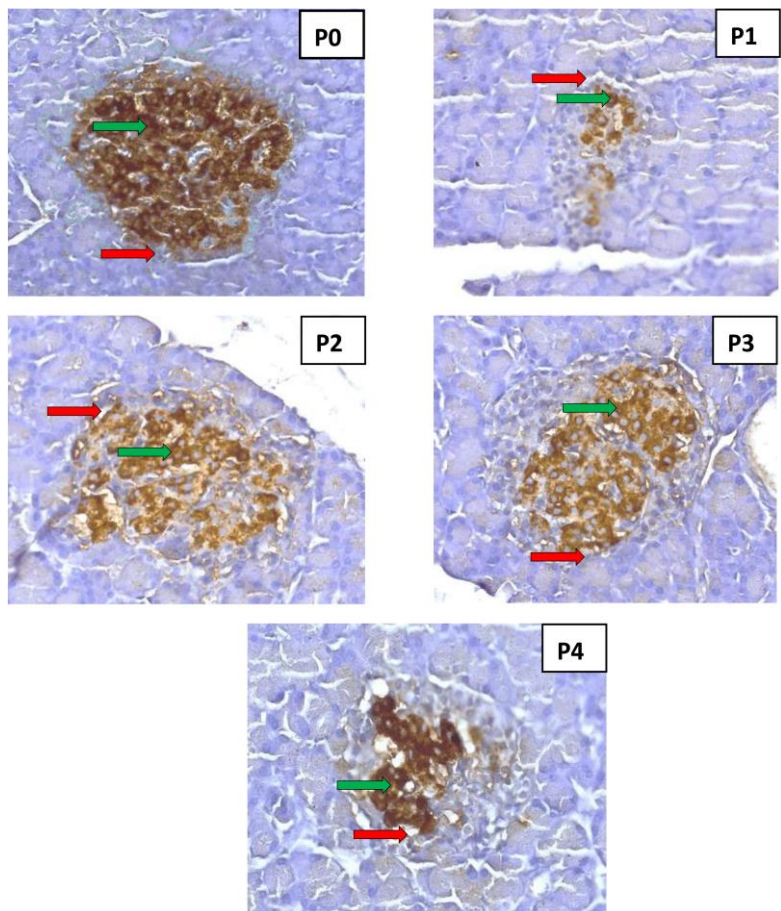


Fig. 2. Histopathological description of the islet of Langerhans in experimental rats examined through immunohistochemistry (IHC) staining after 28 days of treatment with 400x magnification. P0 = Normal; P1 = DM; P2 = DM + 5 ml/kg BW of BT Kombucha; P3 = DM + 5 ml/kg BW of FSB Kombucha, P4 = DM + 5 ml/kg BW of FSB Brew; Green arrow: Pancreatic β -cells that are immunoreactive to anti-insulin; Red arrow: Endocrine cells of the islets of Langerhans.

3.2 Discussion

Faloak (*Sterculia quadrifida* R.Br) stem bark (FSB) brew has been consumed as an herbal drink by the people of Timor Island, East Nusa Tenggara, Indonesia. However, scientific evidence is needed in exploring this drink, especially as a natural therapeutic agent for diabetes mellitus which has not been widely studied yet. To increase the efficacy and consumers' interest in presenting an attractive flavor, FSB brew is used as a kombucha substrate. This study compared the anti-diabetic potential of FSB drink fermented using a kombucha consortium with the FSB brew which had been empirically consumed by the local community.

The use of alternative substrates in kombucha drinks has been extensively explored. However, the use of a local herbal substrate based on faloak stem bark for kombucha was first examined in this study. Shahbazi et al. [22] previously reported that the utilization of cinnamon herbal substrate in the making of kombucha could improve physicochemical, sensory, and antimicrobial activities. In this study, FSB substrate and control BT fermented

using the kombucha consortium showed changes in pH, total acid, and total sugar during 14 days of fermentation. According to Jayabalan et al. [6], yeast hydrolyzes sucrose with the help of the invertase enzyme into glucose and fructose which will then be converted by acetic acid bacteria into organic acids (acetic acid and glucuronic acid). The accumulation of organic acids at the end of the fermentation results in a decrease in pH due to the release of H⁺ ions by microbial metabolism [23]. The variation in parameter values between FSB kombucha and BT kombucha samples is associated with the variation of dominating microorganisms between the two types of kombucha [24]. Thus, the conversion process of the raw material during fermentation also depends on the level of microbial population contained in each kombucha sample.

The increase in the components of phenolic and flavonoid compounds from both kombucha samples for 14 days had an impact on increasing the ability to scavenge DPPH free radicals, as reported in other studies [14, 21, 22]. According to Villarreal-Soto et al. [25], changes in phenol and flavonoid contents were associated with microbial bioactivity. Chakravorty et al. [26] and Bhattacharya et al. [14] added that microbes degrade phenols and flavonoids by enzymes they produce during fermentation. The increase in phenolic and flavonoid compounds in FSB and BT kombucha samples contributed to increasing the ability to scavenge DPPH free radicals. Lulan et al. [1] previously identified phenol and flavonoid components in the extract of faloak (*Sterculia quadrifida* R.Br) stem bark. Therefore, the components of these compounds could be increased by fermentation in this study. The increase in phenols and flavonoids showed a correlation with the increase in antioxidant activity within 14 days of fermentation. The accumulation of increased components of bioactive compounds contributes to its ability to scavenge free radicals. It can be seen in Table 1 that FSB kombucha showed an increase ability to scavenge free radicals from 70.79% on Day 0 to 82.21% on Day 14, while the ability of the control BT kombucha increased from 70.41% to 80.15%; both did not show a significant difference ($p > 0.05$). The dissimilarity in the values of antioxidant activity between both types of kombucha is suspected to be related to the natural content of the bioactive compound components on each substrate and the diversity of the population of microorganisms in degrading the bioactive components, thereby contributing to differences in antioxidant activity.

The high antioxidant properties of FSB kombucha prompted the exploration of its functional activity as a natural therapeutic agent for diabetes mellitus (DM) in this study. The ability of FSB kombucha to scavenge free radicals is presumably able to be used as an alternative to DM therapy due to the effects of free radicals. According to Bhattacharya et al. [14], pathophysiological causes and complications of DM can be associated with free radical exposure. Hyperglycemic conditions in the rats examined in this study were carried out by inducing alloxan to form superoxide ion compounds that can damage pancreatic β -cells and impaired insulin secretion [9, 27]. Polyphenols compounds and organic acids in kombucha manage to increase insulin activity by neutralizing the form of free radicals and pancreas regeneration, also to reduce polysaccharide hydrolysis and sugar assimilation [9].

As an indicator of exposure to free radicals and the presence of endogenous antioxidant activity, MDA levels and SOD activity were analyzed. The data proved that DM control rats showed an increase in MDA levels and a decrease in SOD compared to normal rats. Increased MDA and decreased SOD were also reported in other studies [10, 11]. The opposite outcome was observed in the groups of DM rats given FSB kombucha, BT kombucha, and FSB brew, where they showed the effects of decreasing MDA and increasing SOD activity within 28 days of treatment. The components of phenolic and flavonoid compounds in kombucha and FSB brew contribute to neutralizing free radicals caused by lipid peroxidase and stimulate the activity of the SOD enzyme. According to Budak et al. [28] and Zubaidah et al. [11], phenolic and flavonoid compounds have an

extremely large contribution in counteracting free radicals with a radical scavenging mechanism. In addition, they are able to stimulate the antioxidant activity of the SOD enzyme.

The impact of DM complications often encountered is cardiovascular disease due to dyslipidemia on the lipid profile associated with the presence of free radical compounds [29]. Identical phenomenon was shown in DM rats examined in this study, where there were an increase in TC, TG, and LDL and a decrease in HDL compared to normal rats. The administration of kombucha and FSB brew showed a significant decrease in lipid profile levels (Table 3). Improvements in lipid profiles in DM rats were also observed in other studies done by Aloulou et al. [9], Bhattacharya et al. [14], and Zubaidah et al. [10]. The increase in serum lipid profile levels was associated with the mechanism of electron transfer of hydrogen atoms from the bioactive compounds of kombucha and FSB brew as well as the lipid radical compounds formed by the induction of alloxan. According to Zubaidah et al. [10], phenolic compounds are able to improve fat metabolism by reducing cholesterol absorption, increasing bile acid secretion, and increasing the activity of the lecithin cholesterol acyl transferase (LCAT) enzyme in utilizing free cholesterol into cholesterol esters. Organic acid compounds contained in kombucha contribute to lowering the levels of TC, TG, and LDL by stimulating protein kinases in maintaining lipid balance in the body and inhibiting lipogenesis [21].

Inducing alloxan to DM rats resulted in the destruction of their pancreatic β -cells characterized by changes in the shape and size of the islets of Langerhans and a decrease in the number of cells observed through IHK staining. According to Szkudelski [30], the damage to pancreatic β -cells causes a decreased insulin secretion, thereby increasing blood glucose accompanied by weight loss. This is in line with other studies done by Bhattacharya et al. [14] and Zubaidah et al. [10]. On the other hand, the groups of DM rats given FSB kombucha, BT kombucha, and FSB brew for 28 days showed cell improvement, reduced blood glucose levels, and balanced body weight. The positive changes in DM rats given kombucha and FSB brew were presumably due to the role of polyphenolic compounds. According to Barbosa et al. [31], polyphenolic compounds are able to repair pancreatic β -cells through reducing the development of cell toxicity by donating hydrogen atoms. El-Kordy and Alshahrani, [32] added that flavonoid compounds can provide the ability to regenerate β -cells so that insulin secretion increases.

The results of this study showed that the administration of FSB kombucha, BT kombucha, and FSB brew had a positive impact on the process of repairing pancreatic β -cells, thus allowing insulin secretion to increase and blood glucose to decrease along with normal body weight in alloxan-induced DM rats. The research data indicated that FSB kombucha was more effective against the impacts of these changes than FSB brew. On the other hand, it was comparable to BT kombucha. This is possible due to the high antioxidant activity and the content of polyphenolic compounds in kombucha drinks as a result of the microbial fermentation activity of the consortium. According to Bhattacharya et al. [14], kombucha provides a more effective impact due to the role of bacteria and yeast in producing metabolites that have potential as antioxidants comparable to glibenclamide. Meanwhile, Aloulou et al. [9] stated that the administration of antioxidant-rich kombucha in DM rats was able to reduce ROS-mediated toxicity on insulin-producing pancreatic β -cells. The presence of organic acid compounds promotes the high effectiveness of kombucha drinks. Andlauer et al. [33] explained that the presence of organic acid compounds in form of gluconic acid and glucuronic acid plays a role in detoxifying toxins in the body. In addition, the fermentation process is able to increase anionic mineral components and nutritional elements which also contribute to pathophysiological improvements [14].

This study was the result of experiments in experimental animals, furthermore advanced tests are needed in the form of a clinical trial on human participants suffering from diabetes mellitus to confirm the accuracy and safety of faloak stem bark kombucha (FSB).

4 Conclusion

This study showed that the brew of faloak (*Sterculia quadrifida* R.Br) stem bark (FSB) fermented using a kombucha consortium for 14 days was able to increase the bioactive components and antioxidant activity. The administration of FSB kombucha, black tea kombucha, and FSB brew to alloxan-induced diabetic rats was effectively able to improve oxidative stress status, reduce blood glucose levels, improve serum lipid profiles, and improve pancreatic β -cells. However, the administration of FSB kombucha significantly showed a more effective ability than that of FSB brew. FSB can be used as an alternative substrate in kombucha, especially as a natural therapeutic agent for diabetes mellitus.

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