The Potential of White Tea (Camellia sinensis) and Kelor (Moringa oleifera) in Improving Lipid Profile and Histopathological Features of Pancreas in Streptozotocin-Induced Rats

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ABSTRACT

This study aimed to examine the potential of white tea and kelor in improving lipid profile and histopathological features of the pancreas in streptozotocin-induced Sprague-Dawley rats. The research design was an experimental study with post-test control group design. Catechin content were analysed using High-Performance Liquid Chromatography. Twenty Sprague-Dawley rats (out of 24 rats, 12-week old, 200-300 g) were induced with intraperitoneal injection of streptozotocin at a dose of 40 mg/kg BW. Four groups of rats received one of the green tea (GT), white tea (WT), kelor (K), or the mixture of white tea and kelor (WTK) with a dose of epigallocatechin-3-gallate (EGCG) at 100 mg/kg BW administered orally for 21 days. Measurements of the lipid profile and preparation of histopathological features of the pancreas were performed after the intervention was completed. The results showed that white tea had the highest concentrations of catechin (39.17%), gallic acid (1.09%), EGCG (4.46%), and epicatechin (9.61%) compared to the other tea groups. The TG levels of the WTK group (95±19.35 mg/dl) were significantly different (p<0.05) from the WT and K groups but not significantly different from the normal (105.8±23.89 mg/dl), DM and GT groups. Meanwhile, the HDL levels did not show significant differences in each intervention group (p>0.05). The mean diameter of the islets of Langerhans in the DM group (9.16± 2.56 μm) was significantly different (p<0.05) from the WT (20±8.94 μm), K (17.16±5.26 μm), WTK (18.66±4.17 μm), and N (21.07±8.49 μm) groups but not significantly different from the GT group (14.33±5.24 μm). The histopathological features of the pancreas showed an increase in the diameter of the islets of Langerhans in WT, K, and WTK groups. This study revealed that the mixture of white tea and kelor had the potential to ameliorate triglyceride levels and histopathological features of the pancreas in streptozotocin-induced Sprague-Dawley rat.

Keywords: histopathology, kelor, moringa oleifera, triglycerides, white tea

INTRODUCTION

Diabetes is a chronic metabolic disease characterized by an increase in blood glucose levels, and it causes serious damage to the heart, blood vessels, eyes, kidneys, and nerves (Ali-poor et al. 2012). About 422 million adults lived with diabetes in 2014 compared to 108 million adults in 1980 (WHO 2016). One of the most common complications of diabetes mellitus is dyslipidemia: i.e. an increase in total cholesterol, low-density lipoprotein cholesterol (LDL), and triglyceride (TG) levels as well as a low concentration of high-density lipoprotein cholesterol (HDL) in plasma. The streptozotocin induction in experimental animals will cause damage or atrophy and a decrease in the size of most of the islets of Langerhans when compared to normal rats (Yun et al. 2006).

Diabetes mellitus can be prevented by implementing lifestyle modification. The lifestyle modification, through qualitative nutritional improvement and exercise, is reported to reduce the relative risk of type 2 diabetes by 40-70%. In line with the exercise, the healthy dietary patterns full of antioxidants, active biopeptides, phytoestrogens, and fish as a source of polyunsaturated fatty acids also needs to be applied (Effendi 2013). Some plants that are rich in antioxidant have been reported to have antidiabetic activity
such as white tea (Camellia sinensis) and kelor (Moringa oleifera) (Gupta et al. 2014; Divi et al. 2012; Mbikay 2012; Islam 2011).

Tea is a source of polyphenols, especially flavanols and flavonoids. A part of flavonoids, catechin, is found at concentration of 30-40% in green tea (Reygaert 2014). Catechin, gallic acid, EGCG, and epicatechin belong to flavanol or flavan-3-ol groups which are included in flavonoids. The monomers of flavonoids and their derivatives are widely found in tea leaves (Tsao 2010).

White tea is one of the types of tea obtained from the tea shoots in the form of buds. It does not go through fermentation process; thus, its catechin content is higher than green tea (Textiera et al. 2012). White tea has anti-hyperglycemic activity and it can reduce total cholesterol and LDL levels in serum (Textiera et al. 2012; Islam 2011). It can also limit free radicals by binding them to reactive oxygen species (ROS) (Gupta et al. 2014; Jigisha et al. 2012).

Kelor (Moringa oleifera) - a native plant from India- grows in tropical and subtropical regions and it is also known as a drumstick or horseradish tree. Its leaves are rich in minerals, vitamins, and other essential phytochemicals. Kelor is reported to have antidiabetic (Gopalakrisnan et al. 2016; Abdulkadir et al. 2015; Malki & Rabey 2015; Hemant et al. 2014), antihyperglycemic, and antihypertriglyceridemic activities (Wardani et al. 2015; Geleta et al. 2016).

Based on the information above, this study aimed to examine the potential of white tea and kelor in improving the lipid profile and histopathological features of the pancreas in streptozotocin-induced Sprague-Dawley rats.

METHODS

Design, location, and time
This research was an experimental study with a post-test control group design. The study design used was a completely randomized design (CRD). The study was conducted at the laboratory of the Post-Harvest Research Station, Laboratory of Biology and Chemistry of IPB University’s Diploma Program, Laboratory of Animal Management Unit (LAMU) of the Faculty of Veterinary Medicine of IPB University, and Veterinary Research Station from January to December 2017.

Materials and tools
The main ingredients used were white tea and green tea obtained from the Research Institute for Tea and Cinchona (RITC), Gambung and kelor which was obtained from PT Moringa Organik Indonesia, Blora. The green tea in this study was used as a positive control since there have been many studies on green tea as an antidiabetic agent (Park et al. 2014). The catechin analysis from the steeped tea was performed using High-Performance Liquid Chromatography (HPLC) and the standards used were catechins, gallic acid, epigallocatechin-3-gallate (EGCG), and epicatechin (EC). The standard used had high purity level at 98% and 80% for gallic acid and EGCG respectively. Meanwhile, the catechin standard used in this study was obtained from Sigma Aldrich. The standard solution (1 mg/ml) was prepared by adding 0.05% formic acid in 70% acetonitrile to the standards. The HPLC system consisted of Smart Line HPLC Knauer GmBH with a UV detector (Smart Line UV Detector 2500 A 5140®), Smart Line Dual Pump 1000 V 7603, and Rheodyne Loop A135 sample injector with a 20 µl volume. The column used was Eurosphere C-18 (250×4.6 mm ID, 5µm). The reverse phase HPLC in this study used an isocratic mobile phase elution system which was a mixture of 0.1% orthophosphoric acid, water, acetonitrile, and methanol (14:7:3:1 v/v/v/v) at a pH value of 4 and a flow rate of 1.2 ml/min. Detection was performed at a wavelength of 280 nm (Martono & Martono 2012).

The experimental animals used were 24 male Sprague-Dawley rats aged 12 weeks with a weight of 200-300 g. The rats were obtained from Indoanilab and they had received laboratory animal health certificates. The tools for lipid profile measurement was Lipid Pro meter (Infofia Co., Ltd), Lipid Pro test strips, lancets, and alcohol. The weight of the liver and kidneys were measured by digital scales. The histopathological preparations were made using 10% neutral buffered formalin (NBF) solution, paraffin, and microscope slide. The tools used for hematoxylin-eosin (H&E) staining were tissue cassettes, dehydrator, vacuum machine, and microtome machine.

Procedure

Material preparation. A total of 2 g of each ingredient (white tea, green tea, kelor, and a mixture of white tea and kelor with 1:1 ratio) was steeped in 100 ml of water at 90 °C for five minutes and then filtered using filter paper. The filtrate collected was used to measure the catechin, gallic acid, EGCG, and epicatechin contents.

The measurement of catechin, gallic acid, EGCG, and epicatechin contents. The catechin standard solution was prepared from the standard solution (1 mg/ml) by adding 0.05% formic acid...
in 70% acetonitrile to the standard. The samples to be measured (i.e., green tea, white tea, kelor, and white tea+kelor that had been steeped) were first extracted with 25 ml of HPLC mobile phase, which consisted of 0.1% orthophosphoric acid, water, acetonitrile, and methanol with a ratio of 14:7:3:1 v/v/v/v. The extraction was performed using a sonicator for five minutes. Next, the extracts were filtered using a microfilter (0.45 μm) and then diluted ten times with the mobile phase. Twenty microliters of analytes were then injected into the HPLC. The concentration was calculated using the area of each chromatogram and compared to the standard compounds used in the measurement. The comparison results obtained were then plotted into the standard curve of the linear regression equation (Martono and Martono 2012).

**Streptozotocin induction and experimental animal care.** The experimental animals were adapted for 14 days before the intervention. After passing through the adaptation period, the experimental animals were assigned to the normal group (4 rats) and streptozotocin-induced groups (20 rats). The streptozotocin induction with a dose of 40 mg/kg BW was carried out intraperitoneally after the rats had overnight fasting. The first blood glucose measurement was performed on the 3rd day after streptozotocin induction. The experimental animals were considered as hyperglycemic if their blood glucose levels were ≥126 mg/dl (Chaplin 2016). Twenty hyperglycemic rats were then divided randomly into five groups as follows: 1) diabetes (DM) control group (i.e., the rats induced with streptozotocin without receiving any intervention); 2) the group receiving streptozotocin induction and the steeped green tea (GT); 3) the group receiving streptozotocin induction and the steeped white tea (WT); 4) the group receiving streptozotocin induction and the steeped kelor (K); and 5) the group receiving streptozotocin induction and the mixture of white tea and kelor (WTK). The intervention groups were given the steeped tea or kelor using feeding tubes with an EGCG dose of 100 mg/kg BW. The intervention was conducted for 21 days. The oral gavage was carried out at 9-10 a.m. All groups received standard feed and ad-libitum drinking. With an EGCG dose of 100 mg/kg BW, the total ingredients required were 2.2 g white tea, 7.75 g green tea, 4.13 g kelor, and 4.06 of the white tea and kelor mixture. The steeped tea or kelor sample was concentrated using a rotary evaporator at a temperature of 58-60 °C for 20 minutes so that the tea or kelor could be administered orally to the rats (Afify et al. 2011). Therefore, the volume of the extract given was in the range of 0.5-2 ml/rat. The ethical consideration for the implementation of this study was obtained from the Animal Ethics Committee, Research and Community Service Institute of IPB University Number 66-2017-IPB.

**Measurement of blood glucose levels.** The measurement of blood glucose levels was performed using a glucometer (Nesco®) and glucose strips (Nesco®). The rats’ blood glucose levels were measured after the rats had overnight fasting (12-14 hours). The blood samples were taken from the tip of the rat’s tail using a needle.

**Organ and blood sampling.** After 21 days of intervention, the experimental animals were anesthetized intraperitoneally (IP) with the ketamine-xylazine combination at a dose of 75-100 mg/kg BW and 5-10 mg/kg BW. After the experimental animals were declared dead, the surgery was performed to take the pancreas for histopathological preparations.

**The making of the pancreatic histopathological preparations.** The histopathological preparations were made by performing fixation to pancreatic tissue using 10% NBF solution. The tissue was cut, arranged in tissue cassettes, dehydrated automatically with a dehydrator, dried in a vacuum machine, and blocked with paraffin liquid. The tissue was then cut into 3-5 μm with a microtome machine, and this piece was attached to the microscope slide. The microscope slide was then stained manually with H&E stain. The microscopic observation was performed using the Olympus CH20 microscope.

**Data analysis**

The data of TG, HDL, and diameter of the islets of Langerhans were analysed using normality test (Kolmogorov-Smirnov) and one-way ANOVA. If there were significant differences (p<0.05) in the effect of the intervention, the analysis was continued with the Duncan’s post hoc test.

**RESULTS AND DISCUSSION**

**Catechin, gallic acid, EGCG, and epicatechin contents in *Camellia sinensis* and *Moringa oleifera.*

Table 1 shows that white tea has the highest flavanol content compared to the other types of tea (i.e. green tea, kelor, and a combination of white tea and kelor). The combination of white tea and kelor has higher catechin, gallic acid, and EGCG contents than green tea. The study of Martono and Martono (2012) showed that the gallic

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**Table 1**

| Compound          | White Tea | Kelor | Green Tea | WTK    |
|-------------------|-----------|-------|-----------|--------|
| Catechin (mg/100 g) | 120       | 90    | 100       | 110    |
| Gallic acid (mg/100 g) | 80        | 60    | 70        | 75     |
| EGCG (mg/100 g)    | 100       | 80    | 90        | 95     |
| Epicatechin (mg/100 g) | 50        | 40    | 55        | 60     |

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acid and EGCG contents of the green tea were in the range of 0.45-0.50 g/100 g and 2.08-3.98 g/100 g, respectively. The study conducted by Hilal & Engelhart (2007) indicated that the catechin and EGCG contents of white tea were 13.22 g/100 g and 8 g/100 g, respectively. Meanwhile, the catechin and EGCG contents of green tea were 12.95 g/100 g and 6.75 g/100 g, respectively. The differences in results obtained from various studies can be caused by the catechin content of tea plants influenced by the harvesting, production process, geographical location, growth condition, and preparation (Reygaert 2014).

Chemically, the higher content of the bioactive compounds content in tea, the higher activity of the compounds. However, its metabolism that occurs in the body is not a chemical mechanism but rather a biochemical mechanism. At certain doses, a bioactive component may have antioxidant properties. However, at other doses, the same bioactive component may have prooxidant properties (Rohdiana 2015; Kim et al. 2014).

Triglycerides (TG) and high-density lipoprotein (HDL) levels after the intervention.

After streptozotocin induction, the mean blood glucose levels reached 396.2±85.52 mg/dl. Meanwhile, the blood glucose levels in the normal group were 69.75±13.47 mg/dl. Based on the normality test, the blood HDL and TG levels were normally distributed. Based on ANOVA, the HDL levels after intervention did not show significant differences (p>0.05) in each group while the TG levels showed significant differences (p<0.05).

The lowest TG levels were found in the WTK group (95±19.35 mg/dl) (Table 2). This result was not significantly different from the normal group (105.8±23.89 mg/dl), the DM group (124±6.93 mg/dl), and GT group (138±48.33 mg/dl), but it was significantly different from the WT group (173.5±20.21 mg/dl) and the K group (167.3±44.85 mg/dl).

The studies conducted by Roghani and Baluchnejadmorajad (2010) and Wolfram et al. (2006) showed an improvement in lipid profile in streptozotocin-induced rats in the presence of EGCG intervention. However, the intervention conducted in our study did not cause a significant difference in the decrease of TG levels as compared to the group that did not receive any intervention (DM group). It was interesting that there were significant differences between the group receiving WTK intervention and the groups receiving only white tea or only kelor interventions. The interaction between bioactive components in white tea and kelor was likely to cause the decrease in TG levels compared to the intervention using only white tea or kelor.

Several studies on the interherbal synergism have been widely investigated (Che et al. 2017; Rahma et al. 2017; EGCG: epigallocatechin-3-gallate). The current study further substantiated the complementary and additive effects of bioactive components from different sources in healthy people and patients with diabetes.

### Table 1. Catechin, gallic acid, epigallocatechin-3-gallate (EGCG), and epicatechin contents in 100 g of ingredients

| Types of tea    | Catechin (g) | Gallic acid (g) | EGCG* (g) | Epicatechin (g) |
|-----------------|--------------|----------------|-----------|----------------|
| White tea       | 39.17        | 1.09           | 4.46      | 9.61           |
| Green tea       | 20.46        | 0.36           | 1.29      | 3.73           |
| Kelor           | 5.91         | 0.24           | 2.42      | 0.97           |
| White tea + kelor | 30.98       | 0.69           | 2.46      | 0.56           |

*Rahma et al. 2017, EGCG: epigallocatechin-3-gallate

### Table 2. Plasma triglyceride and high density lipoprotein levels after intervention

| Groups   | HDL (mean±SD) in mg/dl | p-value | TG (mean±SD) in mg/dl | p-value |
|----------|------------------------|---------|-----------------------|---------|
| DM       | 71 ± 4.61              | 0.051   | 124 ± 6.93b           | 0.01**  |
| GT       | 51.5 ± 1.41            |         | 138 ± 48.33b          |         |
| WT       | 49 ± 1.54              |         | 173.5 ± 20.21b        |         |
| K        | 53.75 ± 7.41           |         | 167.3 ± 44.85b        |         |
| WTK      | 57.5 ± 1.60            |         | 95 ± 19.35a           |         |
| Normal   | 46.25 ± 1.24           |         | 105.8 ± 23.89a        |         |

*DM: Diabetes Mellitus; GT: Green Tea; WT: White Tea; K: Kelor; WTK: White Tea + Kelor **ANOVA and then continued with Duncan’s post hoc test (significant at p<0.05). The different superscripts show significant differences. HDL: high density lipoprotein. TG: triglyceride
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2013; Wagner & Mezernich 2009; Berenbaum 1989) and referred as to the isobole method (i.e. a mixture of two or more herbs that produce antagonistic interactions, zero interaction, or synergistic interactions). The interaction between white tea and kelor may produce a synergistic mechanism. The mechanism that may occur due to the synergistic effect is that the combination of multi-extracts will have an impact on various types of different enzyme activity, substrates, metabolites, receptors, ion channels, transport proteins, and ribosomal DNA/RNA. Besides that, the psychochemical and pharmacokinetic interactions between white tea and kelor may increase the solubility of the extract combination (Wagner 2011). The comparison of different combinations of white tea and kelor needs to be further investigated to produce a better lipid profile data.

Histopathological features of pancreas and diameter of the islets of Langerhans in rats after the intervention.

Streptozotocin is a toxic material that can directly cause damage the pancreatic β cells. The diabetogenic mechanism of streptozotocin is DNA alkylation through nitrosourea. The DNA damage will cause activation of poly-(ADP-Ribose) polymerase, which then leads to cellular NAD+ suppression, depletion of the amount of ATP, and it can eventually cause pancreatic necrosis (Szkudelski 2001).

The islet of Langerhans is a multihormonal endocrine micro-organ in the pancreas. The islets appear as a group of round building whose cells are buried in the exocrine pancreatic tissue. The islets of Langerhans cannot be seen with naked eyes. However, if stained with the H&E solution, the islets can be selectively stained (Banjarnahor & Sunny 2012).

The mean diameter of the islets of Langerhans in the DM group (9.16±2.56 µm) was not significantly different from the GT intervention group (14.33±5.24 µm). However, it was significantly different from the WT (20±8.94 µm), K (17.16±5.26 µm), and WTK (18.66±4.17 µm) intervention groups as well as the N group (21.07±8.49 µm). The mean diameter of the islets of Langerhans in the WT intervention group was not significantly different from the N group (Table 3).

A study by Yun et al. (2006) showed that the streptozotocin injection would cause damage or atrophy and the decrease in the size of most of the islets of Langerhans in the experimental animals when compared to normal rats. Yun et al. (2006) also explained that the intraperitoneal administration of EGCG and vitamin E increased the size of the islets of Langerhans.

Hanhieneva et al. (2010) reported that EGCG had a protective effect on β cells. The pancreas is one of the targets of the bioactivity of dietary polyphenols. Plant extract and pure phenolic compounds have been reported to be useful for pancreatic β cell function and insulin secretion in several diabetic experimental animals. However, there has not been a single mechanism in this study that has been identified for the given response.

A study by Ambarwati et al. (2014) reported that kelor intervention with a dose of 500 mg/kg BW in streptozotocin-induced rats for 21 days gave an improved in blood glucose levels. The results of a study conducted by Malki and Rabey (2015) concluded that the administration of kelor with the doses of 50 and 100 mg/kg BW for four weeks in streptozotocin-induced rats gave an improved the histopathological features of the pancreas.

A study conducted by Noor et al. (2017) and Wikanta et al. (2011) used the diameter of the islets of Langerhans as a parameter to assess the improvement of the hyperglycemic rat’s conditions. The improvement was characterized by the increased diameter of the islets of Lang-

| Groups   | The diameter of the islets of Langerhans (mean±SD) (µm) | p-value |
|----------|--------------------------------------------------------|---------|
| DM       | 9.16 ± 2.56 a                                         | 0.010** |
| GT       | 14.33 ± 5.24 ab                                        |         |
| WT       | 20 ± 8.94 bc                                          |         |
| K        | 17.16 ± 5.26 b                                        |         |
| WTK      | 18.66 ± 4.17 b                                        |         |
| Normal   | 21.07 ± 8.49 c                                        |         |

*a*: Diabetes Mellitus; GT: Green Tea; WT: White Tea; K: Kelor; WTK: White Tea+Kelor **ANOVA and then continued with Duncan’s post hoc test (significant at p<0.05). The different superscripts show significant differences.
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The DM group had a smaller diameter of the islets of Langerhans than the other groups, but it was not significantly different from the GT group. The groups receiving white tea, kelor, and the combination of white tea and kelor interventions showed an increase in the mean diameter of the islets of Langerhans that was significantly different from the DM group. The administration of EGCG with the same dose in each intervention group gave different results in increasing the diameter of the islets of Langerhans due to the role of other components (i.e. catechin, gallic acid, and epicatechin) and the interaction between the bioactive components in white tea and kelor, that had the potential to improve the histopathological features of pancreas.

The results of this study showed that EGCG was not the only component that played a role in the improvement of lipid profile (HDL and TG), but the combination of white tea and kelor had the potential to reduce TG levels better than the intervention using only white tea or kelor. White tea, kelor, and WTK have the potential to improve the histopathological features of the pancreas. Further research on the comparison of white tea and kelor and the interaction between white tea and kelor need to be conducted to improve the effect the lipid profile and histopathological feature of the pancreas better.

CONCLUSION

The combination of white tea and kelor have the potential to increase the diameter of the islets of Langerhans and improve the histopathological features of the pancreas better that a single administration of each material.

In the future, a study on different combinations of white tea and kelor, the interaction between white tea and kelor, and the role of these two ingredients in the gene expression involved in diabetes mellitus should be further investigated.
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