



**KOMISI ETIK PENELITIAN
FAKULTAS KEDOKTERAN HEWAN UNIVERSITAS AIRLANGGA
*Animal Care and Use Committee (ACUC)***

**KETERANGAN KELAIKAN ETIK
“ ETHICAL CLEARANCE ”**

No : 1.KE.127.07.2019

**KOMISI ETIK PENELITIAN (ANIMAL CARE AND USE COMMITTEE)
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Penggunaan Simplisia Kayu Manis (*Cinnamon
burmanii*) dan Kembang Bulan (*Tithonia diversifolia*)

PENELITI UTAMA : Hani Plumeriastuti

**UNIT/LEMBAGA/TEMPAT
PENELITIAN** : Penelitian Terapan Unggulan Perguruan Tinggi
Fakultas Kedokteran Hewan Universitas Airlangga

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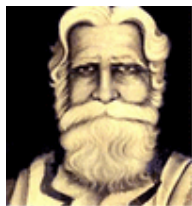
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Sushruta, an ancient Indian Surgeon, whose magnum opus is Sushruta Samhita

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Effect of Cinnamon (*Cinnamomum burmannii*) Bark Oil on Pancreatic Histopathology of white Rats (*Rattus norvegicus*) Induced with Streptozotocin

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Abstract

The purpose of this research was to understand the effect of the cinnamon (*Cinnamomum burmannii*) bark oil (CBO) on the pancreatic histopathology white rat (*Rattus norvegicus*) with diabetes mellitus. The variables taken were the diameter and the number of Langerhans Island in the pancreatic of white male rats which had been induced by streptozotocin (STZ). This research using diabetic rat induced by streptozotocin intraperitoneally with single dose of 45 mg/kg BW. Samples consist of fifty rats were divided into five groups, negative control group (K-) was not induced by streptozotocin, which treatment by giving CMC Na 1%, positive control group (K+) was induced by streptozotocin without CBO, group P1 was induced by streptozotocin and gave CBO 100 mg/kgBW, group P2 was induced by streptozotocin and gave CBO 200 mg/kgBW, group P3 was induced by streptozotocin and gave CBO 400 mg/kgBW daily for 14 days period. The results showed that cinnamon bark oil (CBO) with dose 200 mg/kgBW (P2) was significantly higher ($p < 0,05$) than group P1, group P3 and positive control group (K+). It can be concluded that CBO can be used to improve pancreatic function of STZ induced in diabetic rats

Keywords : *Streptozotocin, Cinnamomum bark oil, Island of Langerhans, Diabetes mellitus, Rats.*

Introduction

Treatment for DM patients is currently mostly done by administering intravenous insulin and oral antidiabetic drugs, such can cause gastrointestinal side effects, hypoglycemic reactions, allergic skin reactions, gastrointestinal disorders and ketoacidosis. Therefore, natural herbal remedies are needed from plants that do not cause side effects. Herbal alternative medicine is one form of treatment therapy that is more affordable to the public, has mild side effects and is easily obtained¹.

Cinnamon is a spice shaped like a bark and is widely used as a spice for cooking cakes and drinks. Cinnamon plant including one of the plants that are often found in the tropics with a number of diverse species. Cinnamon plants that have been studied have antidiabetic effects including *Cinnamomum zeylanicum*, *Cinnamomum burmannii*, and *Cinnamomum cassia*². *Cinnamomum burmannii* is a type of cinnamon that is mostly found in Indonesia³. Cinnamon is a plant that has the effect of hypoglycemia, where the plant contains cinnamic acid and cinnamaldehyde which can increase insulin circulation and are effective in reducing fasting blood glucose levels².

Cinnamon bark oils (CBO) contain cinnamaldehyde and eugenol compounds which have potential as antimicrobial organisms⁴ and cinnamaldehyde compounds resulting from the isolation of cinnamon oil have the potential to enzyme α -glucosidase⁵. This

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enzyme plays a role in the breakdown of carbohydrates into blood sugar, so that the essential oil content can be developed as an antidiabetic compound. Based on the description above, the effect of cinnamon essential oil in repairing pancreatic β cells as antidiabetic has not been proven. Therefore, the authors wish to conduct research on the effects of cinnamon essential oil on the histopathology of the pancreatic Langerhans island in white rats (*Rattus norvegicus*) induced by streptozotocin.

Materials and Methods

The experimental animals used were white male rats (*Rattus norvegicus*), healthy wistar strains aged 2-3 months with a body weight between 150-250 grams totaling 50 animals. The white rat (*Rattus norvegicus*) was first adapted for seven days in the Pharmacology Laboratory of the Faculty of Medicine, Airlangga University. Diabetic white mice were made by streptozotocin-induced at a dose of 45 mg / kg body weight (BW) injected intraperitoneally. This injection was carried out in all treatments except negative controls. Streptozotocin (STZ) is dissolved in a citrate buffer solution with a pH of 4.5. STZ induction was done immediately after making a single dose of 45 mg / kg BW⁶.

White rats (*Rattus norvegicus*) male Wistar strain of 50 animals divided by five groups, each group of ten. Adaptation was done for seven days with the aim to avoid stress in experimental animals. The study were conducted with five treatments including: (K-): A group of white mice that were not induced by STZ and given CMC Na 1%, (K +): A group of STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and given 1% CMC Na, (P1): A group of STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and treated with CBO at a dose of 100 mg / kgBW orally, (P2): STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and treated with CBO at a dose of 200 mg / kgBW orally, (P3): A group of STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and treated with CBO at a dose of 400 mg / kgBW orally^{7,8,9}.

White rat samples that have been made with histopathological preparations using *Hematoxylin* and *eosin* (HE) staining, followed by examination of white rat pancreatic histopathological preparations at the Veterinary Pathology Laboratory

of the Faculty of Veterinary Medicine, Airlangga University, Surabaya.

Results and Discussion

Histopathological observations of Langerhans Island white rats in group K (-) were only given CMC Na 1% solvent, K (+) was induced by streptozotocin 45 mg / kg BW, groups P1, P2 and P3 were treated with CBO, once for 14 days. The dose of CBO given is 100 mg / kg BW, 200 mg / kg BW and 400 mg / kg BW.

The data analyzed are the number of cells and the diameter of the island of Langerhans. Data obtained from observations and calculations on histological preparations in accordance with the treatment, repetition and dosage used. The results of statistical tests using the ANOVA (Analysis of Variance) test showed that there were significant differences ($p < 0.05$) between treatments followed by Duncan's test.

The results were obtained from the calculation of the number of cells and the measurement of the diameter of the island of Langerhans which was carried out using a 400x magnification microscope. the results of observations in the control and treatment groups can be seen in Table 1 and Table 2. The histopathological picture of Langerhans Island can be observed in Figure 1. The results of observations in Table 1 mean the highest number of Langerhans island cells are group P2 and the lowest in group K (+).

Analysis of Variance (ANOVA) statistical test results showed a significant difference ($p < 0.05$). Further test is the Duncan test with the results obtained by the treatment of K (-) significantly different from K (+), P1, and P3. The treatment of K (+) is significantly different from K (-), P2 and P3.

In Table 1 and Table 2 it can be seen that P1 and P3 show significant differences with P2, so to determine the correct dosage in the use of cinnamon essential oil need to compare the three treatments with a negative control group K (-). P1 and P3 statistical test results were significantly different from K (-), but not significantly different from K (+).

The results of microscopic histopathological observations can be seen in Figure 1. P2 group was diabetic group of rats that gave cinnamon essential oil

at a dose of 200 mg / kg BW orally and produced the highest number of cells and diameter compared to other treatment groups, namely 62.50 ± 21.54 and 91.96 ± 12.38 . The cell looks neatly arranged and fills the island of Langerhans. Group P3 is a group of white rats with DM patients who were given orally cinnamon essential oil at a dose of 400 mg / kg body weight or 48.40 ± 16.24 cells. The obtained diameter is 76.54 ± 16.21 . Cells appear to be rare on the island of Langerhans. The number of cells and diameter of the Langerhans island produced was higher than the treatment groups K (+) and P1. The variance analysis test gave significantly different results ($p < 0.05$) on the number and diameter of the Langerhans island K (-), P2 with the treatment groups K (+), P1, and P3.

Hyperglycemia is a condition in which the body cannot control blood glucose levels caused by disruption of insulin secretion by β cells in the pancreatic islets of Langerhans. The process of glucose metabolism is very important to produce energy sources for the survival of cells in the body. However, in a state of hyperglycemia, the body's cells cannot metabolize glucose so the body lacks an energy source. It responds to the body to search for alternative energy, namely from glycogenesis and gluconeogenesis. Both of these processes produce by-products, namely free radicals, which cause damage to cells such as pancreatic β cells^{10, 11}.

B cell death in the Langerhans island can automatically change the structure of the Langerhans island in terms of shape and size. Therefore, the size of the diameter or size of the area can be presented as an indicator of damage and repair of pancreatic organs in hyperglycemic conditions¹². B cell death is very influential on the area of the island of Langerhans, because there are 60% -80% of β cells in the total endocrine cells in the island of Langerhans. This shows that almost all of the endocrine cells contained in the island of Langerhans are β cells¹³. This study was conducted to determine the effect of CBO on increasing the number of cells and the diameter of the pancreatic Langerhans island in white rats induced by streptozotocin. The results of histopathological examination and observation of the number of cells and the diameter of the pancreatic Langerhans island showed that the positive control group (K +) obtained the lowest average compared to other treatments. This is due to this group of streptozotocin-induced white rats 45mg

/ kgBW and only given 1% Carboxymethyl Cellulose Sodium (CMC Na) therapy. CMC Na is inert, safe and does not react with other groups¹⁴.

It has been proven that cinnamaldehyde compound from cinnamon oil isolation against the α -glucosidase enzyme so that it is very potential as an α -glucosidase enzyme inhibitor. IC50 value is a number that shows the concentration of extract (ppm) that can inhibit 50% of the activity of the α -glucosidase enzyme⁵. Compounds are said to be active to inhibit the activity of the α -glucosidase enzyme¹⁵.

The enzyme α -glucosidase plays a role in the breakdown of carbohydrates into blood sugar. Inhibition of α -glucosidase in mammalian intestine can reduce blood glucose levels from metabolism of polysaccharides or oligosaccharides. Slowing the absorption of blood glucose causes a reduction in postprandial hyperglycemia to prevent chronic complications of diabetes mellitus such as retinopathy, neuropathy, neuropathy and microvascular as well as macrovascular problems¹⁶. The group that plays a role in inhibiting the enzyme α -glucosidase is the cinamoil group. The component of cinnamon oil containing cinamoil group is cinnamaldehyde¹⁷. Cinnamaldehyde is a compound that has a functional group of aldehydes and benzene ring conjugated alkenes. The study of Ping et al (2010) states that cinnamon oil has an antidiabetic effect in mice¹⁸. Components contained in cinnamon oil are cinnamaldehyde (62-75%), eucalyptol, copaene, naphthalene, eugenol, benzaldehyde. Cinamaldehyde contained is useful as antidiabetic¹⁹.

Zhu R et al (2017) states that cinnamaldehyde shows a decreased effect of sugar on test animals through increased sugar release and improvement of insulin sensitivity in adipose tissue and muscle tissue, increases glycogen synthesis in the liver, improves pancreatic islets, slows down gastric emptying time, and improves gastric emptying kidney disorders due to diabetes and brain damage²⁰. The mean cell count and diameter of the white rat Langerhans island increased in P2 group compared to P1 and P3. This is likely due to metabolic disorders or biotransformation of the body. The aim of cinnamaldehyde metabolism is to change non-polar cinnamaldehyde into polar, so that they can be secreted by the kidneys or bile. This process can cause active

cinnamaldehyde to become inactive. However, some become more active, less active and even become toxic²¹. The speed of metabolism can increase the intensity and length of service of the cinnamaldehyde. This metabolic rate may vary in each individual. A decrease in metabolic rate will increase the intensity and prolong the service life of the cinnamaldehyde and possibly increase the toxicity of the cinnamaldehyde²².

Table 1. Mean cell counts of Langerhans island in white rats (*Rattus norvegicus*)

Treatment	Mean Cell Number ± SD
K(-)	60.27 ^b ± 7.96
K(+)	34.20 ^a ± 6.64
P1	37.05 ^a ± 11.96
P2	62.50 ^b ± 21.54
P3	48.40 ^{ab} ± 16.24

Note: a, b different superscripts in the same column show significant differences (p <0.05).

Table 2. Mean diameter of Langerhans island in white rats (*Rattus norvegicus*).

Treatment	Mean Diameter (µm) ± SD
K(-)	87.79 ^b ± 3.61
K(+)	58.83 ^a ± 8.11
P1	60.91 ^a ± 17.18
P2	91.96 ^b ± 12.38
P3	76.54 ^{ab} ± 16.21

Note: a, b different superscripts in the same column show significant differences (p <0.05).

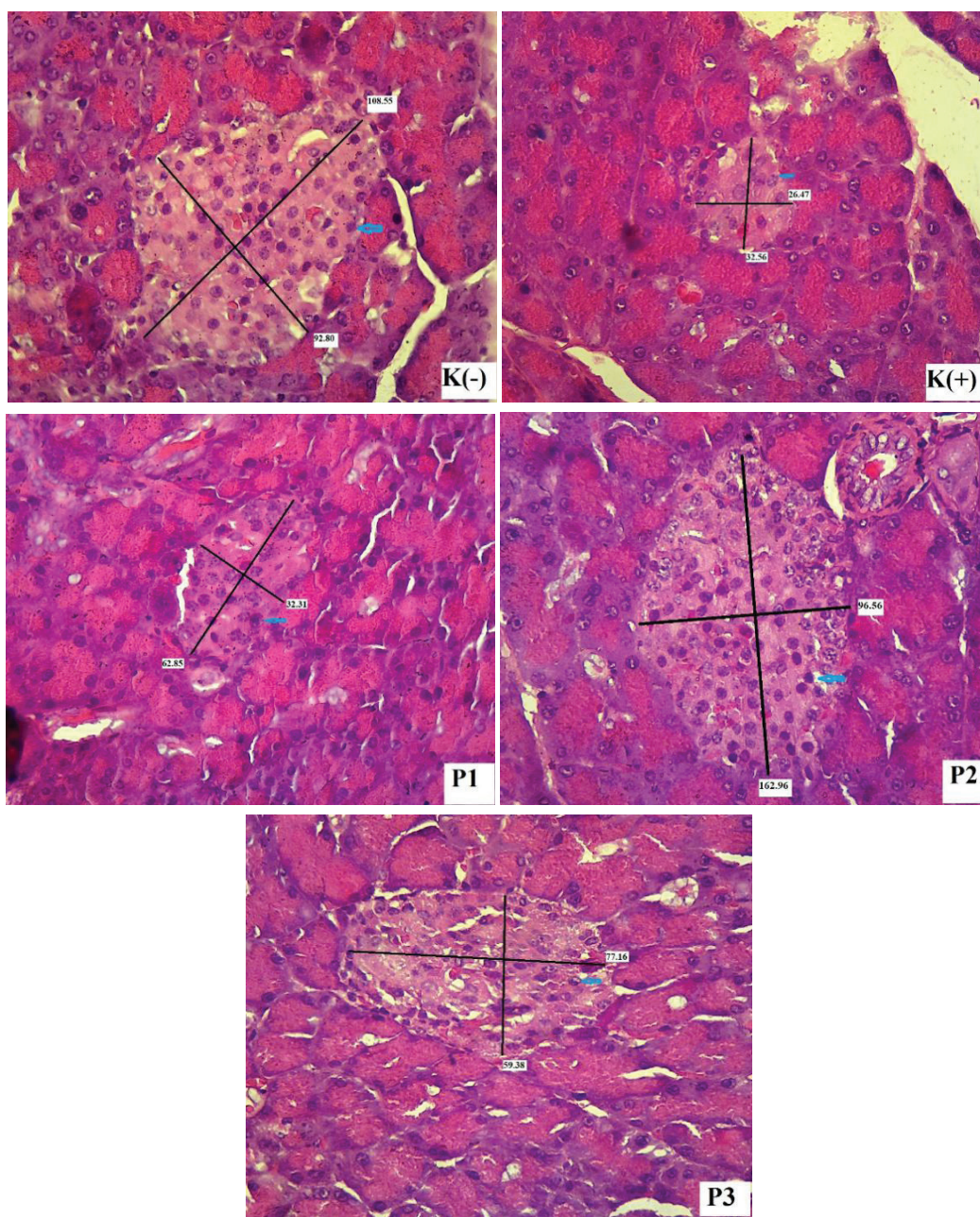


Figure 1. Histopathological picture of Langerhans Island white rat with 40 0x magnification with HE staining at treatment K (-), K (+), P1, P2, P3. on Langerhans Island Cell of Pancreas.

Conclusion

After conducting research on the effect of cinnamon bark oil (CBO) at a dose of 100 mg / kg, 200 mg / kg and 400 mg / kgBW for 14 days, it can be summarized that CBO can increase the number of cells and expand the diameter the pancreatic Langerhans island of white rat (*Rattus norvegicus*) induced by streptozotocin. Therefore, the effect of administration of CBO can be

used as an alternative medicine to increase the number of Langerhans island that produce insulin hormone.

Designation :

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Ethical approval : Ethical clearance was taken and passed from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Airlangga University. Certificate ethic number : 1.KE.127.07.2019.

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