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RESEARCH ARTICLE

Effect of Cinnamon *(Cinnamomum burmannii)* Bark Oil on Testicular Histopathology in Streptozotocin Induced Diabetic Wistar Male Rats

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Abstract

The purpose of this study was to determine the effect of cinnamon bark oil (CBO) on testicular histopathology. The variables taken were the diameter of the seminiferous tubules and the number of leydig cells in the testes of white male rats (*Rattus norvegicus*) which had been induced by streptozotocin. Twenty white male rats adult Wistar strains aged 2-3 months with an average weight of 150-250 grams were used in this study. The rats were divided into five experimental groups (4 rats each group) treated with a combination of streptozotocin and cinnamon bark oil (CBO) which were designed as follows: K(-), K(+), P1, P2, and P3. P is white rat injected with streptozotocin with a single dose of 45 mg / kg body weight intraperitonially, followed by therapy. K(-) without streptozotocin and only given drug solvent, K(+) given drug solvent, P1 treated with CBO 100 mg / kgBB orally, P2 treated with CBO 200 mg / kgBB orally, P3 treated orally with CBO 400 mg / kg orally for 14 days. White rat animals were then mutilated and testicular organs were taken for histopathological preparations using HE staining, then the diameter of the seminiferous tubules and the number of Leydig cells were calculated. Data were analyzed with One Way ANOVA and followed by Duncan's test. The results showed that streptozotocin had the potential to reduce the size of the seminiferous tubule diameter and the number of Leydig cells, while CBO at a dose of 400 mg / kgBW could increase the size of the seminiferous tubule diameter and the number of Leydig cells. It can be concluded that CBO can be used to reduce testicular dysfunction induced in diabetic rats.

Key words: *Cinnamon bark oil, Seminiferous tubule diameter, Leydig cells, Streptozotocin, Male rat.*

Introduction

Diabetes mellitus (DM) is a systemic disease caused by chronic metabolic disorders characterized by high blood sugar levels accompanied by impaired carbohydrate, lipid and protein metabolism as a result of insulin function insufficiency. Broadly speaking DM is divided into two types namely, DM type 1 and Type 2 DM [1]. According to Mihardja et al [2], Indonesia is one of the 10 countries that have the greatest number of people with diabetes. The World Health Organization (WHO) estimates that by 2030 the number of sufferers will surge to 366 million. Indonesia ranks 4th in the highest number of diabetics after India, China and the United States. DM sufferers reach 8.4 million in 2000 and is expected to increase to 21.3 million in 2030 [3].Streptozotocin (STZ) is a chemical commonly given to experimental animals to produce DM in these animals, because it causes damage to pancreatic beta cells [4]. STZ as an indicator of DM (Diabetagon) because it is seen from the working mechanism of the STZ itself. STZ can work directly on pancreatic ß cells because STZ enters ß cells through glucose transporters

(GLUT 2) and causes DNA alkylation. DNA damage induces poly activation ADP-rybosylation, which causes NAD + and cellular ATP. Increased dephosphorylation ATP after STZ induction produces a substrate for xanthine oxidase catalysis reaction which produces superoxid radicals. As a result hydrogen peroxide and hydroxyl radicals are also formed.

Furthermore, STZ frees many toxic substances from nitric oxidants that inhibit aconitase activity and play a role in DNA damage, which ultimately occurs apoptosis and necrosis of pancreatic ß cells [5]. High blood glucose levels or hyperglycemia in people with DM play a role in cell damage by increasing Reactive Oxygen Species (ROS) which can cause tissue oxidative stress which ultimately damages the mitochondrial membrane [6].

Mitochondrial membrane damage due to increased ROS can occur in various tissues including tissues in the reproductive system. The mechanism of damage that occurs in the reproductive system begins with an increase in ROS that causes loss of potential function of the mitochondrial membrane, which results in damage to blood vessel endothelium so that the provision of nutrients is reduced to seminiferous tubular tissues and interferes with the process of spermatogenesis [7].

Cinnamon plant is a species of the genus Cinnamomum with the family Lauraceae, in the form of woody plants commonly known as spices [3]. Cinnamon can be a source of antioxidants because it contains many compounds such as eugenol, safrole, cinnamaldehyde, tannin, and calcium oxalate [8]. According to the research of Yuliarto et al [9], cinnamon (*Cinnamomum burmannii*) bark oil products using water vapor distillation method obtained cinnamaldehyde compound (37.12%), p-Cineole (17.37%), Linalool (8.57%), and Benzyl benzoate (11.65%).Cinnamaldehyde and linalool compounds have been reported as one of the antioxidant compounds [10].

Based on research from Ngadiwiyana et al [11] cinnamaldehyde compounds resulting from the isolation of cinnamon essential oil have the potential for α-glucosidase enzymes that can be developed as antidiabetic compounds. Based on the introduction above, the author would like to conduct research on the antioxidant effect of cinnamon bark oil (CBO) on the number of Leydig cells and the diameter of the seminiferous tubules in the testes of white male rat (*Rattus norvegicus*) Wistar strains DM models through STZ induction.

Materials and Methods

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. The experimental animals used in this study were white male rats Wistar strains aged 2-3 months with an average weight of 150-250 grams, which were induced by STZ and treated with cinnamon essential oil. Aspects observed in this study were the diameter of the seminiferous tubules and the number of white rat testicular Leydig cells.

Diabetes Mellitus Induction

Diabetic white rat were made by induction using STZ with a single dose of 45 mg / kg BW injected intraperitoneally. STZ was dissolved in a citrate buffer solution with a pH of 4.5. Preparation of STZ solution was made by dissolving 162 mg of STZ in 9 mL of 0.1 M citrate buffer (pH 4.5), so that every 0.5 mL contained 9 mg of STZ. This injection is performed on all experimental animals except experimental control animals. Before the STZ induction, animals try to fast for 12 hours, but drinking is still given. After being induced, then wait for 72 hours to assess fasting blood sugar levels. After giving STZ induction, to avoid side effects and the risk of sudden hypoglycemic, sucrose or dextrose solution is given 10% for 12-24 hours [12].

Research Procedure

White rats (Rattus norvegicus) male 20 Wistar strains were divided into five groups, each group of four each, as follows: (K-); A group of white rat that were not induced by STZ and given a drug solvent. (K +); Group of white rats induced by STZ in a dose 45 mg / kg body weight. (P1); Group of white rats induced by STZ at a dose of 45 mg / kgBW and treated with CBO at a dose of 100 mg / kgBW. (P2); Group of white rats induced by STZ at a dose of 45 mg / kgBW and treated with CBO at a dose of 200 mg / kgBW. (P3); Group of white rats induced by STZ at a dose

of 45 mg / kgBW and treated CBO at a dose of 400 mg / kgBW. STZ induction was done once in the study given intraperitonially. STZ induction is done on the eighth day or one day after the adaptation period, then wait for 72 hours to assess fasting blood sugar levels. Provision of cinnamon essential oil is given once a day orally using a gastric sonde for fourteen days.

Histopathological observations made on testicular histopathological preparations of white rats with group K (-) were only given a drug solvent, K (+) was induced by streptozotocin 45 mg / kg BW and given a drug solvent, groups P1, P2 and P3 induced by streptozotocin and treated with CBO, once for 14 days. The dose of CBO given is 100 mg / kg body weight, 200 mg / kg body weight and 400 mg / kg body weight.

Results and Discussion

Data analyzed were seminiferous tubule diameter and number of Leydig cells. Data obtained through observation and calculations on histological preparations in accordance with the treatment, repetition and dosage used. Histopathological observation using scoring method on the diameter of Seminferus tubules and the number of Leydig cells of white rats on five seminiferous tubule fields. The scoring results obtained were then averaged and analyzed using the SPSS for Windows 23. The statistical test results used the ANOVA (Analysis of Variance) test which showed that there were significant differences ($p < 0.05$) between treatments followed by Duncan's test.

The results were obtained from the measurement of the seminiferous tubule diameter and the calculation of the number of Leydig cells carried out using a microscope with a magnification of 100x to measure the diameter of the seminiferous tubules and 400x magnification for the calculation of the number of Leydig cells.

The results of observations in the control and treatment groups can be seen in Table 1. Graphic illustration the mean seminiferous tubule diameter and Leydig cell count calculation can be observed in Fig.1 and Fig 2, while the histopathological picture of seminiferous tubule diameter and Leydig cell count can be observed in Fig. 3 and Fig.4.

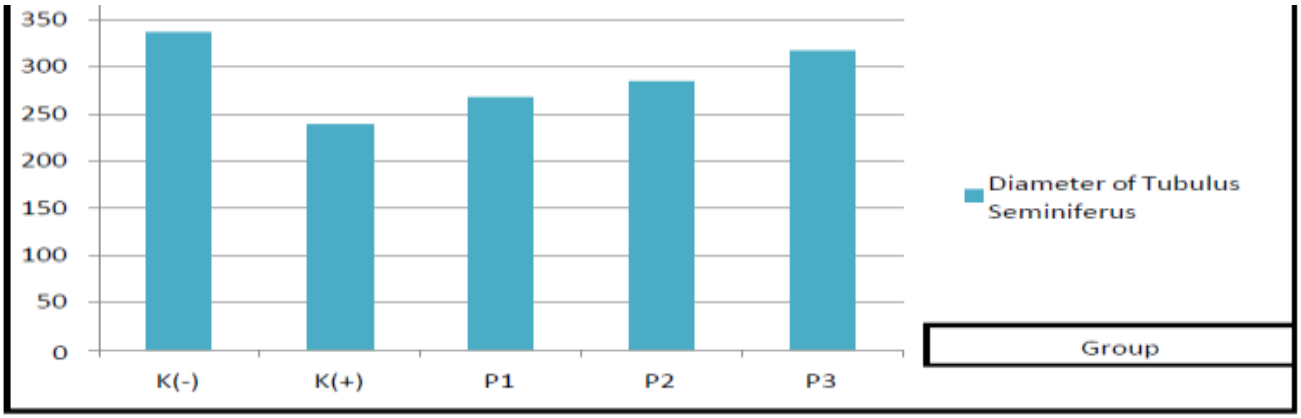

Figure 1: The mean diameter of the seminiferous tubules in the white male rat testis in various treatments

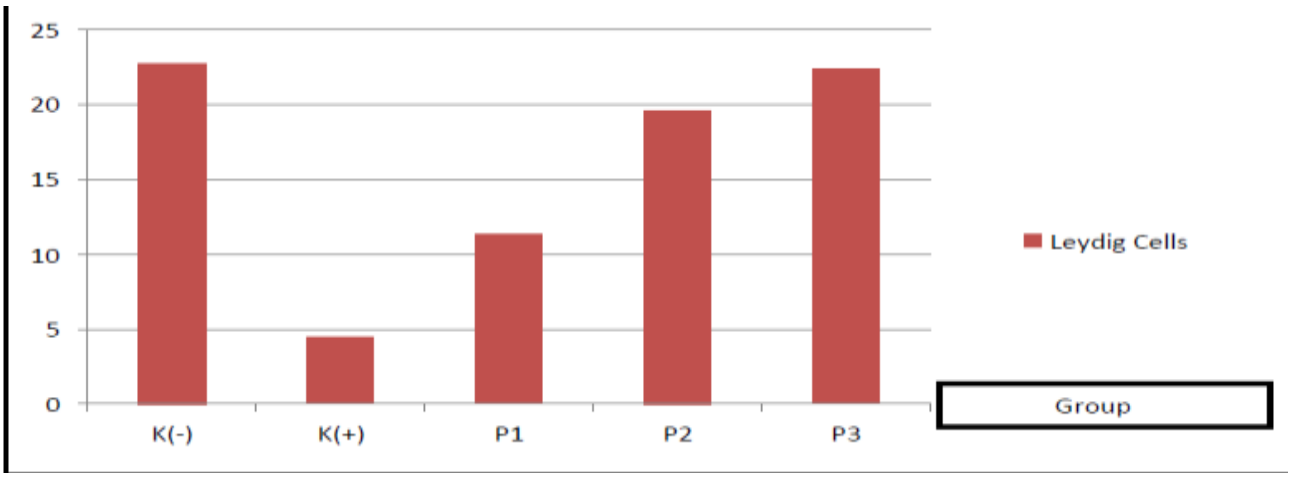

Figure 2: The mean number of Leydig cells in the white male rat testis in various treatments

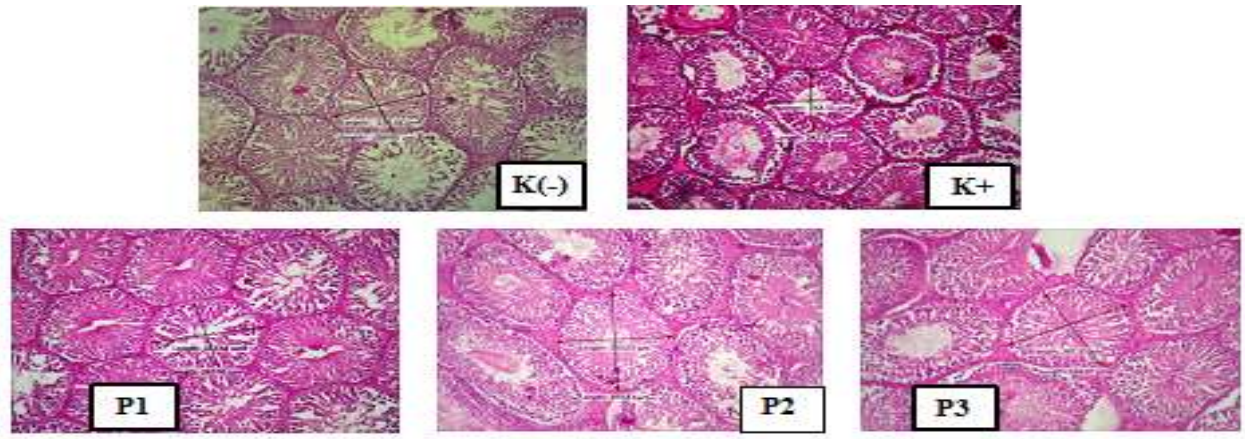

Figure 3: Histopathological picture of the seminiferous tubule diameter in the testicles of white rats with a magnification of 100x with the staining of HE in the treatments K (-), K (+), P1, P2, P3

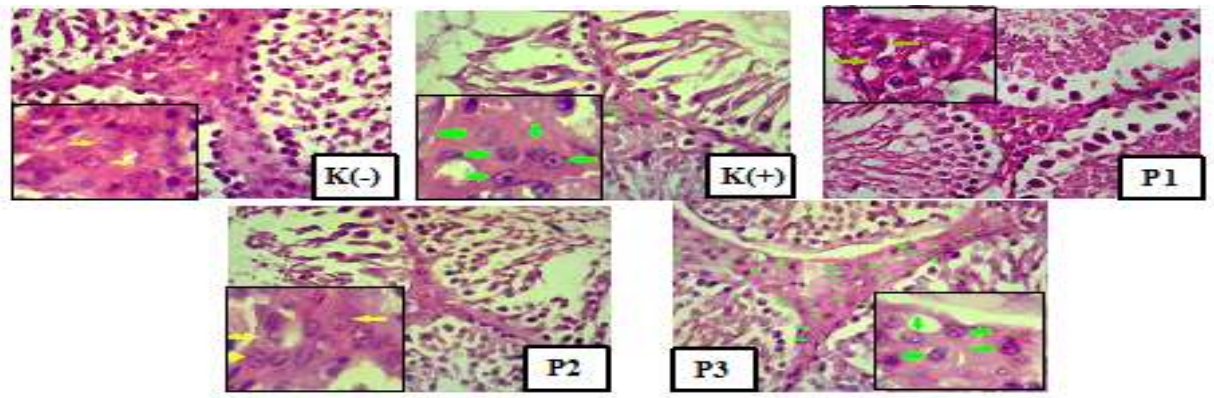

Figure 4: Histopathological picture of the number of Leydig cells in testicles of 400x magnified white rats with HE staining in the treatments K (-), K (+), P1, P2, P3

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 ($p < 0.05$) with K (+), P1, and P2. The treatment of K (+) was significantly different ($p < 0.05$) with K (-), P1, P2 and P3. In the results of Table 1, it can be seen that the negative control K (-) with the highest value does not show a real difference with P3 but shows a real difference ($p < 0.05$) with K (+), P1, P2. The positive control K (+) was significantly different ($p < 0.05$) with K (-), P1, P2 and P3. The treatment of P1 on the diameter calculation was significantly different ($p < 0.05$) with K (-), K (+) and P3, but not significantly different ($p > 0.05$) from P2. P1 treatment on cell count calculation showed significantly different ($p < 0.05$) with K (-), K (+), P2 and P3. The P2 treatment on the diameter calculation shows a real difference ($p < 0.05$) with K (-), K (+) and P3, but not significantly different ($p > 0.05$) from P1. P2 treatment on cell count calculation showed significantly different ($p < 0.05$) with K (-), K (+), P1 and P3. P3 treatment showed significantly different ($p < 0.05$) with K (+), P1 and P2, but not significantly different ($p > 0.05$) from K (-).

Table 1: Average Tubular Diameter of Semifer and Number of Leydig Cells

Group	Diameter of tubulus seminiferus	Number of Leydig cells
K(-)	336.05^{c} µm ± 17.91	22.70^{d} ± 0.74
K(+)	238.98^{a} µm ± 18.76	4.45^{a} ± 1.10
P1	267.38^{b} µm ± 8.88	11.35^{b} ± 1.43
P2	285.19^{b} µm ± 15.44	19.55^{c} ± 2.14
P3	317.26^{c} µm ± 11.05	22.35^{d} ± 0.44

Note: a, b different superscripts in the same column show significant differences ($p < 0.05$)
Information:
K (-): As a negative control a 2ml oral drug solvent was given.
K (+): As a positive control intraperitoneal injection with streptozotocin 45 mg / kgBW + 2ml of oral drug solvent.
P1: Peroral projection with streptozotocin + essential oils with a dose of 100 mg / kgBW orally 2ml.
P2: Peroral projection with streptozotocin + essential oils with a dose of 200 mg / kgBW orally 2ml.
P3: Peroral projection with streptozotocin + essential oil with a dose of 400 mg / kgBW orally 2ml.

Streptozotocin (STZ) has been known to have the ability to damage pancreatic β cells through DNA alkylation, which results in Diabetes Melitus (DM) with the consequence of hyperglycemia. Condition hyperglycemia triggers an increase in Reactive Oxygen Species (ROS) in all cells of the body, including the reproductive system. According to the results of research Sulistyoningrum et al [13], stated that rat suffering from diabetes decreased the diameter of the seminiferous tubules and some of the seminiferous tubules had a low epithelium. Hyperglycemia is a condition of increased blood sugar so that it causes oxidative stress to increase it causes a decrease in FSH and LH secretion [14, 15].

According to the research of Yama et al [16], the decrease in seminiferous tubule diameter is thought to be caused by inhibition of Luteinizing Hormone (LH) secretion which functions to stimulate growth and number of Leydig cells and lack of testosterone and Follicle Stimulating Hormone (FSH) secretion which causes seminiferous tubular atrophy.

This can be proven in the research of Inoue et al [6] that, glucose levels high blood pressure or hyperglycemia in people with DM play a role in cell damage by increasing ROS which can cause oxidative stress tissue which ultimately damage the mitochondrial membrane. Free radicals will damage the cell membrane, mitochondria, and endoplasmic reticulum, resulting in an increase Ca2 + cytosol.

Increased Ca2 + cytosol will activate the enzyme phospholipase, protease, endonuclease, and ATPase which causes a decrease in phospholipids, disruption of membrane and cytoskeleton proteins, DNA fragmentation, and decreased ATP. These conditions will initiate cell death [17].The content of cinnamaldehyde in cinnamon is an antioxidant agent that can fight the formation of ROS by activating Nuclear factor-erythroid-2 related factor 2 (Nrf2).

Nrf2 signal is a signal that can prevent the formation of ROS in a state of hyperglycemia. In addition Nrf2 can also maintain the level of Nitric Oxide (NO) which is a vasodilator agent in blood vessels [18]. The content of vitamin C in cinnamon functions as an antioxidant because it effectively captures free radicals, especially ROS or reactive oxygen compounds [19]. As a free radical scavenger, vitamin C can react directly with superoxide anions, hydroxyl radicals, singlet oxygen and lipid peroxide. As reductant, ascorbic acid will donate one electron to form non-reactive semidehydroascorbate and subsequently undergo a disproportionation reaction to form dehydroaskorbate which will degrade to form oxalic acid and treonic acid. Because of the ability of vitamin C as an inhibitor of free radicals, vitamin C is very important in maintaining the integrity of cell membranes [19].

C. burmanii bark is used as a spice and for the production of essential oils. Cinnamon bark oil (CBO) is one of them the most important essential oil. Although there is no evidence about the cinnamaldehyde effect, which is the main components of CBO used in this study include the structure of the testes It has been reported that consumption of plant extracts contains cinnamaldehyde effectively protects pig lymphocytes against oxidative DNA damage [20]. Oil and other products C. zeylanicum has been reported to cause decreased testicular lipid peroxidation levels, and increased concentrations of LH, FSH, and testosterone [21, 22].

weight of reproductive organs; sperm count and motility; antioxidant activity in healthy animals [23, 24]. In addition, long term CBO administration has been proven to protect testes, epididymids, additional sex organs, and spermatozoa, and to reduce testicular apoptosis against reproductive toxicity induced by carbon-tetrachloride prevent oxidative stress [25]. In this study, CBO administration for Streptozotocin (STZ) induced rats leads to significant an increase in blood sugar levels that affect the testicular histopathological severity lesions, apoptosis compared with only rats given drug solvent.

The P3 group was compared to the only group with STZ induction treated with CBO which was able to neutralize testicular severity due to STZ.Possible explanation for this improvement after the administration of CBO prevention occurs imbalance in the antioxidant systems by the CBO. In this study clearly that STZ causes a decrease in reproductive function male organs with the occurrence of testicular apoptosis, as well as

inducing histopathological testicular tissue lesion. Disorders of the testicles which are reproductive systems are potentially linked to STZ induced increased peroxidation rate and lipid reduction in the activity of antioxidant enzymes. Other than that, the findings of this study clearly show that consumption of CBO protects male reproduction rats organs, tissues, and cells resist structural and functional damage of STZ by its antiperoxidative effect. However, physiological and metabolic enhancements changes cannot be observed, due to inhibitory effects STZ on other organ damage is not examined in this study.

Conclusion

The findings mentioned above suggest that increased use of essential oils from Cinnamon bark can reduce sides effect of diabetes mellitus on testicular histopathological damage. To our knowledge, this is the first report that showed that cinnamon bark oil (CBO) can reduce testicular dysfunction induced in diabetic rats. CBO can contribute to balanced oxidant-antioxidant status and provide therapeutic options that are beneficial for reducing related testicular injuries.

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