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Improved Quality of Spermatozoa Mice (*Mus musculus*) Model of Diabetes Mellitus Type 1 After Being Given Cinnamon Bark Methanol Extract (*Cinnamomum burmanii* Bl.)

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Abstract

Diabetes mellitus has to do with male fertility, a hormone disorder that affects spermatogenesis. This study aims to determine the effect of cinnamon bark methanol extract (Cinnamomum burmani) on the quality of diabetes mellitus mice spermatozoa. This study used a complete randomized design with 4 treatments and 3 repeats. 22 mice were divided into 4 groups. Mice have injected with streptozotocin dose of 0.1 mL and observed blood sugar levels were, if sugar levels increased, they are given cinnamon bark methanol extract at a dose of 250 mg/kg BB and 500 mg/kg BB, and blood sugar levels are measured. After that, the mice are dissected for observation of spermatozoa's morphology, viability, and motility after administration of the extract. The results showed that cinnamon bark methanol extract at a dose of 250 mg/kg BB and 500 mg/kg BB can reduce the number of abnormal spermatozoa and increase the viability and motility of diabetes mellitus mice spermatozoa. This indicates that cinnamon bark extract as an antioxidant has a positive effect in maintaining structure and development, as well as the function of spermatogenesis cells so that in the presence of these active substances, the number of seed cells that experience developmental failure, degeneration, death due to free radicals can be suppressed or reduced.

Keywords: *Cinnamon, Morphology, Motility, Viability of Spermatozoa*

Abstrak

Diabetes mellitus mempunyai hubungannya dengan fertilitas pria adalah gangguan pada hormon yang mempengaruhi spermatogenesis. Penelitian ini bertujuan untuk mengetahui efek ekstrak methanol kulit batang kayu manis (Cinnamomum burmani) terhadap kualitas spermatozoa mencit diabetes mellitus. Penelitian ini menggunakan Rancangan acak lengkap dengan 4 perlakuan dan 3 kali ulangan. Mencit sebanyak 22 ekor dibagi menjadi 4 kelompok. Mencit diinjeksi streptozotocin dosis 0,1 ml dan diamati kadar gula darah, apabila kadar gula sudah mengalami peningkatan maka diberi ekstrak methanol kulit batang kayu manis dengan dosis 250 mg/kg BB dan 500 mg/kg BB dan dilakukan pengukuran kadar gula darah. Setelah itu mencit dibedah untuk pengamatan morfologi, viabilitas dan motilitas spermatozoa setelah pemberian ekstrak. Hasil penelitian menunjukkan bahwa ekstrak methanol kulit batang kayu manis dengan dosis 250 mg/kg BB dan 500 mg/kg BB dapat menurunkan jumlah spermatozoa abnormal dan meningkatkan viabilitas dan motilitas spermatozoa mencit diabetes mellitus. Hal ini mengindikasikan bahwa ekstrak kulit batang kayu manis sebagai antioksidan berpengaruh positif dalam memelihara struktur dan perkembangan, serta fungsi sel-sel spermatogenesis, sehingga dengan adanya zat aktif tersebut maka jumlah sel-sel benih yang mengalami kegagalan perkembangan, degenerasi, kematian akibat radikal bebas dapat ditekan atau dikurangi.

Kata Kunci: *Kayu Manis, Morfologi, Motilitas, Viabilitas Spermatozoa*

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INTRODUCTION

Diabetes mellitus is a chronic disease and will persist for life.[1] Ironically, the disease exerts a systemic influence that leads to the occurrence of functional disorders of the body in general. One of the impacts caused by this disease is in the form of impaired male reproductive function related to a decrease in sperm quality which is one of the causes of infertility in men.[2]

Diabetes mellitus has to do with male fertility, a hormone disorder that affects spermatogenesis.[3] In addition, there are abnormalities in the process of spermatogenesis itself followed by the presence of ejaculation disorders. In diabetics, there is a significant decrease in testosterone levels accompanied by a decrease in LH and FSH levels.[4]

Spermatogenesis is a fundamental process in the male reproductive system that involves a series of high-level genetic and epigenetic events within germ cells that play an important role in converting spermatogonia into spermatozoa ((Irdalisa, 2021) by being controlled by gonadotropin hormones, namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH).[5]

The quality parameters of spermatozoa that are important to determine the fertility of a man include viability, number, morphology, and motility of spermatozoa. The motility and speed of motion of spermatozoa are closely related to the fertilization process. If the spermatozoa swim or move very slowly then the total number of spermatozoa that fertilize the ovum is too small. To approach the ovum, spermatozoa must swim quickly and move like a spiral, which is what is referred to as the capacitating motility pattern.[6]

The use of herbal medicine in treating diabetes mellitus has been used for generations because, in addition to the relatively small side effects, the price is also more economical. This traditional treatment places more emphasis on subjective complaints. Traditional medicine is one of the alternatives in medicine because its side effects are considered smaller and cheaper than modern medicine.[7]

Cinnamon (*Cinnamomum burmani*) is a spice in the form of bark that is commonly used by Indonesians in their daily lives. Apart from being an enhancer of the taste of cooking and baking, the cinnamon plant is known to have various properties. Cinnamon contains chemical compounds in the form of Metylhydroxy Chalcone Polymer and Tyrosine Phosphatase IB (PTP1B), phenols, terpenoids, tannins, and saponins which are sources of antioxidants.[8] The compounds Metylhydroxy Chalcone Polymer and Tyrosine Phosphatase IB (PTP1B) can serve as insulin substitutes by protecting the beta pancreas cells of mice.

The purpose of this research was to prove that cinnamon bark methanol extract (*Cinnamomum burmani*) can

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improve the quality of the spermatozoa mice model of diabetes mellitus type 1.

METHODOLOGY

Research Type

This research is a laboratory experimental research.

Tools and Materials

The tools used in this study were mice cages, digital Ohaus Scales, Erlenmeyer, sonde tools, experimental animal surgical tools consisting of scalpels, tweezers, scissors, needles, candle tables, object glass, Petri dishes, microscopes, haemocytometers, hand counters, and digital cameras.

While the ingredients used are cinnamon bark, methanol, equates, STZ (streptozotocin), 22 male mice, aluminum foil, 70% alcohol, filter paper, cotton, tissue, formalin, feed mice, and PAM water, Giemsa solution, 70% alcohol, NaCl 0.9, equates, and rice husks.

Implementation of Research

Trial Animal Setup

Before the study begins, a place for keeping experimental animals is first prepared, namely cages (plastic tubs), chaff, and places for eating, drinking, and feeding mice. After that, acclimatization is carried out in the laboratory for 1 week.

Mice are divided into 4 groups, namely group I: the negative control group (not diabetes) consisting of mice tails, group II: the positive control group i.e. mice injected with streptozotocin but not given cinnamon bark methanol extract, group III: mice group injected with streptozotocin and given cinnamon bark methanol extract dose 250 mg/kg BB and Group IV: the group of mice injected with streptozotocin and given cinnamon bark methanol extract at a dose of 500 mg/kg BB. Each group consists of 5 mice.

Injection Solution Preparation

Before injection, STZ was dissolved in citrate buffer with pH 4.5 (5.25 g of citrate buffer solution (Na_2HNO_4) weighed and 22.5 mL of aqueous), homogenized then inserted 39.8 mg STZ was so that the final concentration of streptozotocin solution was obtained was 22.5 mg/mL STZ. STZ solution is injected intraperitoneally as much as 0.1 mL per head. Mice with glucose levels exceeding 200 mg/dl are considered diabetes.[9]

Test Material Preparation

The bark of the cinnamon stick is taken and dry air at room temperature. After drying, it is mashed with a blender and the smooth powder is weighed. The manufacture of cinnamon bark methanol extract is carried out using the maceration method using ethanol solvent. After obtaining the liquid extract, the extract is then evaporated using a rotavapor at a temperature of 40^{o}C until a concentrated extract of methanol is obtained from the bark of the cinnamon stick.

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Testing Procedure

Before the treatment, cinnamon bark was given to blood glucose levels to ensure that 12 mice had diabetes. Measurement of blood glucose levels is carried out by taking mice blood through the tail which is first cleaned with alcohol. Then the blood is dripped on the glucometer strip and put in the glucometer to read the glucose level.

The test procedure is carried out by testing 4 groups each consisting of 3 mice. The extract was given for 2 weeks and in the last week of testing, blood sugar levels were measured using glucose.

Spermatozo Quality Observations

Observations on the quality of spermatozoa mice include morphology, viability, and motility carried out when administering STZ and after administration of cinnamon bark methanol extract.

Data Analysis

The observational data were analyzed with Analysis of Variance (ANOVA) with the SPSS 16.00 program. The difference between treatments using the BNT test with a confidence level of 0.05%.

RESULTS AND DISCUSSION

Abnormal Spermatozoa Count

The results of observations of spermatozoa morphology in diabetic Mellitus mice and given cinnamon bark extract (*Cinnamomi burmanii*) where what is calculated is the abnormal morphology of spermatozoa can be seen in Table 1.

Table 1. The average number of abnormal spermatozoa of diabetes mellitus mice after administration of cinnamon bark methanol extract.

No.	Treatment	Average Viability of spermatozoa (%) ± SD
1	Negative control	2,33 ± 1,00[a]
2	Positive control	80,33 ± 3,00[b]
3	Dosage 250 mg/kg BB	26,33 ± 2,52[c]
4	Dosage 500 mg/kg BB	16,67 ± 1,52[d]

Description: Superscripts with the same letter do not differ markedly (P > 0.05).

Based on Table 1, shows that in the negative control group the average number of abnormal spermatozoa was 2.33. The average number of abnormal spermatozoa in the positive control group was 80.33. In the group of diabetic mice treated with cinnamon bark methanol extract before dosing 250 mg/kg BB, the average number of spermatozoa was 26.33. Meanwhile, in the diabetic mice group treated with cinnamon bark methanol extract at a dose of 500 mg/kg BB, the average number of abnormal spermatozoa was 16.67.

The decrease in normal spermatozoa during STZ injection is caused by an increase in primary

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abnormalities due to toxins present in streptozotocin. STZ makes the production of superoxide and Nitrite Oxide (NO) in mitochondria increase, further activating protein kinase C (PKC) and the formation of advanced glycosylated end-products (AGEs) which will both interfere with cell function β.[10]

Reactive Oxygen Species (ROS) will damage the integrity of DNA in the spermatozoa nucleus so that it will induce cell apoptosis.[11] Apoptosis of such cells causes changes in the morphology of spermatozoa, especially at the time of spermatogenesis. The results showed that the administration of streptozotocin caused the occurrence of abnormal spermatozoa, namely on the head, neck, and tail. Abnormalities in the head, neck, and tail in this study are thought to have occurred at the time of the process of spermatogenesis.[12,13]

Spermatogenesis can occur through several stages of division. In the early stages, the spermatogonia will change to primary spermatocytes, then to secondary spermatocytes, and spermatids. Before a spermatid becomes spermatozoa there is a phase that the spermatid passes through called the spermiogenesis phase. This phase consists of the Golgi, cap, acrosome, and maturation phases aimed at forming a normal morphology of spermatozoa consisting of the head, neck, and tail. This disorder can be caused by hormonal consequences, free radicals, and foodstuffs.[14]

According to Prastowo,[15] abnormal abnormalities of the head and tail are characterized by a condition where spermatozoa only have the head, tail, and neck. Abnormalities in this state are thought to occur primarily or secondary, abnormalities occur primarily due to disturbances during the process of spermatogenesis, while secondary abnormalities occur due to damage to spermatozoa during their passage inside the epididymis. The head and tail of spermatozoa are connected by a cell membrane allowing separation during cell movement and cytoplasmic displacement.[16] In spermatozoa that have abnormalities in the posterior part of the head, sometimes a perfect membrane is not formed so that contact with the tail basal is less strong. This is due to damage to the spermatozoa membrane by ROS. In addition, according to Zulfa,[17] lipid peroxidation of unsaturated fatty acids on the head and neck of spermatozoa causes changes in the morphology of spermatozoa.

The administration of cinnamon bark methanol extract in the research showed a decrease in the number of abnormal spermatozoa. The decrease in the average of these abnormal spermatozoa is thought to be due to the content contained in the bark of cinnamon sticks such as flavonoids. The function of most flavonoids in our body is as an antioxidant.[18] Flavonoids are natural antioxidants that can act as

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hydroxyl radical reducers (*OH), superoxides (O2*-), and peroxyl radicals (ROO*).[19] It also contains 313 ppm of tocopherol which dampens 96% of free radicals at a concentration of 12 ppm. Tannins are astringent, bitter-tasting plant polyphenols that can bind and precipitate proteins.

Viability of Spermatozoa

The results of observations on the percentage viability of spermatozoa mice after administration of cinnamon bark methanol extract can be seen in Table 2.

Table 2. The average percentage of viability of spermatozoa mice diabetes mellitus after administration of cinnamon bark methanol extract.

No.	Treatment	Average Viability of spermatozoa (%) (%) ± SD
1	Negative control	68,00 ± 1,00[a]
2	Positive control	22,00 ± 3,00[b]
3	Dosage 250 mg/kg BB	53,67 ± 2,52[c]
4	Dosage 500 mg/kg BB	60.33 ± 1,52[d]

In Table 2, it can be seen that the average viability percentage of spermatozoa mice is 68.00%. In the positive control group, the average percentage of spermatozoa viability was 22.00%. The average percentage of spermatozoa viability in the diabetic mice group treated with cinnamon bark methanol extract dose 250 mg/kg BB was 53.67%. Meanwhile, in the group of diabetic mice treated with cinnamon bark methanol extract at a dose of 500 mg/kg BB, the average percentage of viability of spermatozoa mice was 60.33%.

STZ injection in the study led to a decrease in the percentage of viability (viability of spermatozoa). This can be seen in the average viability percentage of spermatozoa mice in the negative control group was higher when compared to the average percentage of mice viability in the positive control group, the dose group was 250 mg/kg BB and 500 mg/kg BB before being given cinnamon bark methanol extract, where the percentage of viability was lower.

With the low percentage of viability, it is suspected that fertilization will not occur because spermatozoa die before fertilizing the egg. Spermatozoa on their way to vas deferens are not all able to maintain their lives so some die. Decreased viability of spermatozoa can occur due to the presence of obstacles in the epididymis as a place of maturation of spermatozoa that occur due to reduced testosterone hormone. Testosterone is necessary for the viability of spermatozoa in the epididymis.[20] The disruption of hormonal work due to streptozotocin causes the life force of

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spermatozoa to decrease so that many spermatozoa die.[21]

Free radicals contained in STZ will affect Sertoli cells which act as determinants of spermatozoa cell reproduction and a source of nutrition for spermatozoa cells.[22] Free radicals will cause the occurrence of lipid peroxidation of cell membranes and damage the organization of cells. This cell membrane is very important for receptor function and enzyme function so lipid peroxidation results in a complete loss of cellular function.[23]

Giving cinnamon bark methanol extract can increase the percentage of viability of spermatozoa. This can be seen by increasing the percentage of viability of spermatozoa mice given cinnamon bark methanol extract at doses of 250 mg/kg BB and 500 mg/kg BB, while in the positive control group the percentage of spermatozoa viability decreased. The increasing percentage of spermatozoa viability after being given cinnamon extract is caused because cinnamon bark has natural antioxidants such as flavonoids, saponins, and tannins that can provide spontaneous and rapid stimulation power to the hypothalamus. The way antioxidants enter the body is through blood circulation to the hypothalamus and the rest of the body. The hypothalamus will produce the hormone GnRH (Gonadotropin Releasing Hormone) which will then stimulate the anterior pituitary to produce the hormones LH and FSH.[24]

Spermatozoa motility

The application of cinnamon bark methanol extract in this study can improve the motility of mice spermatozoa. The average number of spermatozoa mice during the study can be seen in Table 3.

Table 3. Average motility of spermatozoa mice diabetes mellitus after administration of cinnamon bark methanol extract

No.	Treatment	Average Motility of spermatozoa (%) (%) ± SD
1	Negative control	68,33 ± 1,16[a]
2	Positive control	18,67 ± 2,52[b]
3	Dosage 250 mg/kg BB	53,00 ± 2,00[c]
4	Dosage 500 mg/kg BB	60.33 ± 1,53[d]

In Table 3, it can be seen that the average number of spermatozoa motility in the negative control group was 68.33%. In the positive control group, the average motility of spermatozoa mice was 18.67%. In the group of diabetic mice treated with cinnamon bark methanol extract at a dose of 250 mg/kg BB, the average amount of motility of spermatozoa mice was 53.00%. Meanwhile, in the group of diabetic mice treated with cinnamon bark methanol extract at a dose of 500 mg/kg BB, the average

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motility of spermatozoa mice was 60.33%.

STZ injection in this study also led to a decrease in the motility of spermatozoa mice. The number of spermatozoa motility in the normal control group was greater when compared to the number of spermatozoa motility in the mice group given streptozotocin. STZ will cause inhibition of the energy supply for the process of spermatogenesis. Energy for the motility of spermatozoa is supplied in the form of adenosine triphosphate synthesized by mitochondria on the caudal body. So if there is damage to the mitochondrial membrane, it will be able to interfere with the motility of the spermatozoa. Streptozotocin will cause weight loss of the epididymis because it can stimulate the formation of estrogen in the body which will increase estrogen levels.[25,]

The epididymis is a long circular channel leading into the vase deferens that enlarges de the ampule of the vas deferens.[26] An epididymis is a place of maturation and storage of spermatozoa The epididymis is used to transport spermatozoa coming from the testes to be taken to the vas deferens. The proximal part of the epididymis duct is a place for the absorption of fluid secreted by the testicles and then the maturation of spermatozoa, while the distal part works as a place to store spermatozoa.[27]

Spermatozoa that leave the testicles are not yet fully capable of movement. As it passes through the epididymis, the sperm maturates and gets motility and capacity for fertilization. Sperm motility is enhanced by relaxants that may be produced by the prostate. Sperm maturation in epididymis includes morphological, histochemical, biochemical physiology, biophysical, and metabolic changes.[28]

Giving cinnamon bark methanol extract at a dose of 250 mg/kg BB and 500 mg/kg BB can improve the quality of mice spermatozoa. In the dose group of 250 mg/kg BB, the average amount of motility of spermatozoa mice before the administration of the extract was 43.33 and after the administration of cinnamon bark extract increased by 53.00. Meanwhile, in the dose group of 500 mg/kg BB, the average amount of motility of spermatozoa mice before administration of the extract was 42.67 and increased after administration of cinnamon bark methanol extract by 60.33.

Cinnamon bark extract contains antioxidant compounds such as flavonoids, eugenol, tannins, saponins, and methyl hydroxy chalcone polymer (MHCP). Flavonoids as antioxidants (AH) give hydrogen atoms quickly to free radicals (R*, ROO*) or convert them to a more stable form, while antioxidant radical derivatives (A*) have a more stable state than free radicals. Flavonoids can stimulate cells β the pancreas to produce and secrete insulin.[29] Elevated insulin levels can

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trigger IGF–1 synthesis in the liver that increases the effects of LH and FSH on Leydig cells and Sertoli cells. Such increases can trigger an increase in testosterone and ABP levels used in the process of spermatogenesis. The process of spermatogenesis that is not inhibited will produce spermatozoa in a higher quantity.[30,31]

Flavonoid compounds and saponins also inhibit the metabolism of Low-Density lipoprotein (LDL) in secondary atherosclerosis lesions through inhibition of LDL oxidation in the endothelial and stop the proliferation of smooth muscle cells (SMCs) stimulated by oxidized LDL.[32] In addition, it reduces the toxicity of oxidized LDL to endothelial cells, smooth muscle cells, and macrophages. Thus inhibiting the thickening of the lining of the vascular wall, which consists of fat, foam cells, muscle cells, and matrices. Then the narrowing of blood vessels will be reduced so that blood flow as a nutrient distributor in the reproductive organs is not hampered and the quality of sperm is not disturbed (sperm count and motility). Blockage of blood vessels (arterial and venous incompetence) results in the flow of nutrients for spermatogenetic cells that develop in the seminiferous tubules will be disrupted, which further affects spermatogenesis and the quality of the sperm produced.[33,34]

CONCLUSION

From the results and discussion, it can be concluded that giving cinnamon bark methanol extract can affect the improvement of the quality of spermatozoa mice model of diabetes mellitus type 1. Cinnamon bark methanol extract dose of 500 mg/kg BB is more effective in lowering the number of abnormal spermatozoa and increasing the percentage of viability and motility of spermatozoa mice (*Mus musculus*) with Diabetes Mellitus.

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