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Potential of Combined Insulin Leaf (*Smallanthus sonchifolius*) and Noni Fruit (*Morinda citrifolia* L.) Extracts in Reducing Blood Glucose Level and Spermatogenic Cells Improvement in Diabetic Mice

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Abstract: Elevated blood glucose levels are a hallmark of diabetes mellitus, which can lead to reproductive disorders, particularly affecting spermatogenic cells. This study aimed to evaluate the effectiveness of insulin leaf extract, noni fruit extract, and their combinations in reducing blood glucose levels and their impact on the number of spermatogenic cells in diabetic mice. A total of 36 male mice were divided into 12 treatment groups, including a normal control group, a negative control group (induced with alloxan 120 mg/KgBW), a positive control group (alloxan + metformin), and nine treatment groups with various doses of individual and combined extracts. Blood glucose levels were measured using Accu-Chek, and testicular histology was prepared following laboratory standards. Data were analyzed using the ANOVA test followed by the Duncan post hoc test. Results showed that the combination of insulin leaf extract at 150 mg/KgBW/day and noni fruit extract at 62.5 mg/KgBW/day was the most effective in reducing blood glucose levels. Additionally, the combination of 125 mg/KgBW insulin leaf extract and 50 mg/KgBW noni fruit extract significantly increased the number of spermatogenic cells. Thus, the combination of insulin leaf and noni fruit extracts shows potential as a natural antidiabetic therapy and for restoring spermatogenic cell function in diabetic conditions.

Keywords: Diabetes Mellitus; Mice; Morinda citrifolia L; Smallanthus sonchifolius; Spermatogenic Cells.

Introduction

Diabetes Mellitus (DM) is a disease characterized by hyperglycemia. DM conditions that are not managed properly will result in various complications. This condition is mainly based on the occurrence of microangiopathy in DM sufferers. Manifestations of complications that arise in DM conditions include cerebrovascular disease, cardiovascular disease, retinopathy, nephropathy, neuropathy, and can even manifest in the reproductive organs (Farmaki et al., 2020). Blood glucose levels in DM sufferers are at levels that exceed normal limits, namely ≥126 mg/dl for fasting blood glucose levels and ≥200 mg/dl for random blood glucose levels (Goyal et al., 2020).

DM complications in reproductive organs are often complained of by men. Clinically, DM in the complication phase can cause erectile dysfunction and decreased sperm quality. Histologically, this condition is characterized by atrophy and reduced diameter of the seminiferous tubules and reduced number and

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abnormalities of spermatozoa cells (He et al., 2021). In animal models of DM with alloxan monohydrate induction at a dose of 125 mg/kgBW for 21 days, there was an increase in seminiferous tubule degeneration. The longer DM occurs, the more extensive the damage will be. Degeneration of the seminiferous tubules is characterized by a reduced number of initial cells, evenly distributed Sertoli cells, and a decreased number of spermatogonia cells (Kotian et al., 2019). All of these conditions will have an impact on the spermatogenesis process.

Spermatogenesis is facilitated by the presence of ABP (Androgen Binding Protein) which is a testosterone-binding glycoprotein. Testosterone from Leydig cells binds to AR (Androgen Receptor) in Sertoli cells that produce ABP and inhibin which helps the spermatogenesis process (Smith & Walker, 2014). ABP is secreted by Sertoli cells into the lumen of the seminiferous tubules so that testosterone is transported into the lumen. A decrease in the number of sperm produced is a sign of an increased level of abnormality in the spermatogenesis process. In this regard, in the seminiferous tubules of DM rats, apoptosis in germ cells (spermatogonium and spermatocytes) was found to increase (Liu et al., 2024)

Metformin is an antihyperglycemic drug of the biguanide class, which is widely used for the control therapy of Type II Diabetes Mellitus. Metformin has a mechanism of action by lowering blood glucose concentrations without causing hypoglycemia. Metformin tends to have a small hypoglycemic effect, but has a fairly high gastrointestinal effect of >10% (Sopianti, 2020). In addition to medical treatment, there has also been much research on the therapeutic effects of several herbal agents. Some herbal agents that are thought to be useful in the treatment of DM include Insulin Leaves (*Smallanthus sonchifolla*) and Noni Fruit (*Morinda citrifolia*), Bay leaves (*Syzygium polyanthum* (whigt) Walp), crown of the gods (*Phaleria macrocarpa* (Scheff). Boerl), leaves of the gods (*Gynura sealum* (Lour). Merr), spoon leaves (*Plantago major*.L.), iler (*Coleus scutellarioides* (L) Benth), and so forth (Pang et al., 2019).

Insulin leaf plants and noni fruit have been shown to have active chemicals such as fructooligosaccharides, carbohydrates and flavonoids that can cause a decrease in blood glucose. Flavonoids act as antioxidants that can capture free radicals such as RNS and ROS. Flavonoids can repair damaged tissue because they are associated with phenolic OH groups. Flavonoids are known to act as antidiabetics because they can regenerate Langerhans islet cells (Cahyana & Adiyanti, 2021). Insulin leaves contain flavonoids of the type myricetin-3-O-a-L-rhamnoside (Delazari & Barbalho, 2014). Myricetin is reported to have benefits as a strong antioxidant, anticancer, antidiabetic, anti-inflammatory and liver protector (Zhang et al., 2019). Myricetin can also increase motility and viability of male gametes (Arruda et al., 2022). This study aims to examine the effect of administering Insulin Leaf Extract (*Smallanthus sonchifolla*), Noni Extract (*Morinda citrifolia* L.) and a combination of both extracts on spermatogenic cells in DM mice.State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Method

Preparation of experimental animals

This study used 36 male mice as experimental animals obtained from the Center for Veterinary Farma (PUSVETMA) Surabaya. The age of the mice used was 3 months with a weight of 25-27 grams. Before being used for research, the mice were acclimatized for 1 week. The mice were kept and given food and drink according to laboratory standards.

Extract preparation
Insulin leaf extract

Insulin leaves were air-dried to obtain insulin leaf simplicia. The simplicia was ground by blending and sifting to obtain insulin leaf powder 100 grams of insulin leaf powder was macerated with 1000 ml of 80% alcohol for 3 days and stirred every day. The extract was separated by filtration using filter paper (Whatman). Furthermore, the filtrate was concentrated using a rotary evaporator at a temperature of 40^0 C.

Noni fruit extract

The ripe noni fruit is thinly sliced and air-dried, so that the noni fruit simplicia is obtained. Furthermore, the noni fruit simplicia is mashed by blending and then sifted to obtain noni fruit powder. 100 grams of noni fruit powder is macerated using 80% alcohol as much as 1000 ml for 3 days and stirred every day. Furthermore, it is filtered using filter paper (Whatman). The filtrate obtained is concentrated using a rotary evaporator at a temperature of 400 C.

Measurement of blood sugar levels in mice

Blood sugar measurements in mice are carried out 3 times, namely 1. initial blood sugar levels, 2. after the mice are injected with alloxan in order to obtain diabetic test animals. (mice are said to be diabetic if they have blood sugar levels of 126 mg / dl) and 3. diabetic mice that have been treated. The procedure for measuring blood sugar is as follows: the tip of the mouse's tail is disinfected using alcohol, then the tip of the tail is injured and the blood that comes out is dripped onto the

glucometer test strip (authochek). The blood sugar level is read on the numbers listed on the device.

Animal Treatment

The mice used were 36 mice divided into 12 groups. The treatment of mice was as follows: P1; control mice (K0), P2: mice injected with alloxan at a dose of 120 mg/Kg BB for 3 days (K-). P3. Mice were injected with alloxan at a dose of 120 mg/Kg BB for 3 days then given metformin at a dose of 1.3 mg/20 gr mice for 35 days (K+). Then the other mice (27 mice) were injected with alloxan at a dose of 120 mg/Kg BB for 3 days, then divided into 9 groups with the following treatments: P4, P5, P6, each given insulin leaf extract (*Smallanthus sonchifolius*) at a dose of 250 mg/Kg BB, 300 mg/Kg BB, and 350 mg/Kg BB. Treatment P7, P8, P9, each given treatment of noni fruit extract (*Morinda citrifolia* L) 100 mg/Kg BB, 125 mg/Kg BB and 150 mg/Kg BB; P10, P11, P12, each given treatment of combination of both extracts, namely insulin leaf extract (*Smallanthus sonchifolius*) 125 mg/kg BB + noni fruit extract (*Morinda citrifolia* L) 50 mg/kg BB; insulin leaf extract (*Smallanthus sonchifolius*) 150 mg/kg BB + noni fruit extract (*Morinda citrifolia* L) 62.5 mg/kg BB; insulin leaf extract (*Smallanthus sonchifolius*) 175 mg/kg BB + noni fruit extract (*Morinda citrifolia* L) 75 mg/kg BB. The extract was given for 35 days. On the 36th day, the blood sugar levels of the mice were measured and killed, and surgery was performed to isolate the testes for making testicular histology preparations.

Preparation of testicular histology

Testicular histology preparations were made according to standard laboratory procedures. The testes were fixed in formalin, dehydrated in a series of graded concentrations of ethanol, cleaned using xylol 1, 2 and 3. Then embedded in paraffin wax. Then cut at 4 µm using a microtome and stained with hematoxylin-eosin, then observed under a microscope. Testicular histology preparations were observed by counting the number of spermatogenic cells including spermatogonia, spermatocytes and spermatids using a microscope. The collected data were analyzed using one-way ANOVA and Duncan test with the help of SPSS version 22.

Result and Discussion

Blood Sugar Levels After Alloxan Induction and After Extract Treatment

The results of statistical analysis using one way anova obtained data that there was an effect ($p < 0.05$) of administering insulin leaf extract (*Smallanthus sonchifolius*), noni fruit extract (*Morinda citrifolia* L) and a combination of both extracts at various doses on the blood glucose level in diabetic mice that induced alloxan. Duncan analisis test are presented in the Figure 1.

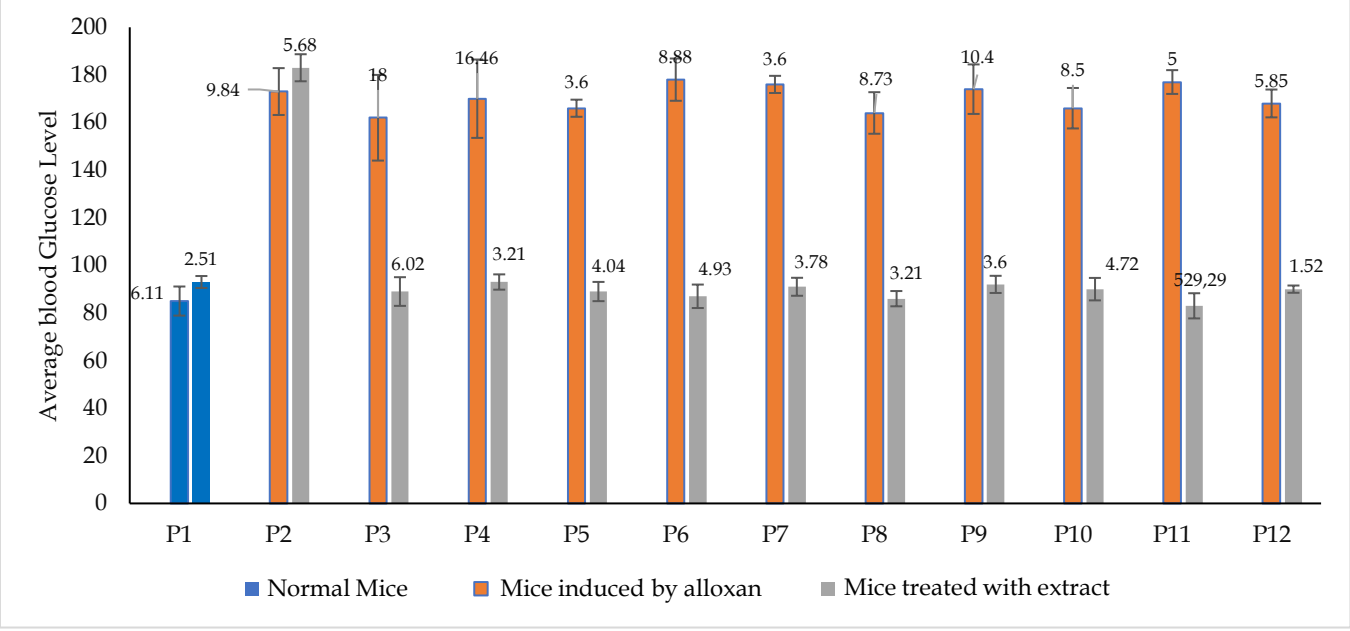

Figure 1. Average blood sugar results of mice after being given alloxan and after extract treatment: P1 control; P2 Negative control; P3 Positive control; P4 Insulin leaf extract 250mg/KgBW; P5 Insulin leaf extract 300mg/KgBW; P6 Insulin leaf extract 350mg/KgBW; P7 Noni fruit extract 100mg/KgBW; P8 Noni fruit extract 125mg/KgBW; P9 Noni fruit extract 150mg/KgBW; P10 Combination of insulin leaf extract 125 mg/kg BW + noni fruit extract 50 mg/KgBW; P11 Combination of insulin leaf extract 150 mg/kg BW + noni fruit extract 62.5mg/KgBW, and P12 Combination of insulin leaf extract 175 mg/kg BW + noni fruit extract 75mg/KgBW

The results of the study showed that mice (*Mus musculus*) that had been induced with 120 mg/kgBW alloxan via IP (Intra Peritoneal) experienced hyperglycemia as indicated by an increase in blood sugar levels ≥ 126 mg/dl (Setiadi et al., 2020). Alloxan is one of the common diabetogenic agents that is often used to assess the antidiabetic potential of pure compounds and plant extracts in studies involving diabetes. Alloxan and streptozotocin (STZ) are the most widely used in diabetes studies. Alloxan-induced diabetes is a form of insulin-dependent diabetes mellitus that occurs due to the administration or injection of alloxan in animals. Alloxan is a urea derivative compound that is often written as 5,5-dihydroxyl pyrimidine-2,4,6-trione with the molecular formula C4H2N2O4 with 142.06 as its relative molecular mass. Alloxan is often used as a reference to determine the possibility of antidiabetic in studies discussing diabetes (Macdonald & Mohammed, 2018). Through the redox cycle, highly reactive hydroxyls will be formed which can cause rapid damage to pancreatic β cells. ROS will cause fragmentation of Deoxyribo Nucleic Acid (DNA) so that DNA becomes damaged, this process will cause necrosis of pancreatic β cells and death of pancreatic β cells so that insulin production will decrease.

In insulin leaves, the content of fructooligosaccharides, flavonoids, smallanthaditerpenic acid, octadecatrienoic acid and Smallanthaditepenic acid A, B, C, D in 10 insulin leaves can lower blood glucose levels. Fructooligosaccharides contained in insulin leaves can modulate metabolic syndrome and dyslipidemia by reducing cholesterol absorption in the small intestine. The content of phenol, chlorogenic, caffeonylquinic, ferulic which are antioxidants in patients with Diabetes Mellitus (DM) can improve pancreatic β cells, because antioxidants are important active components in the regulation of glucose metabolism (Arambas & Vidakovic, 2022).

Morinda citrifolia L contains bioactive components such as flavonoids, triterpenes, triterpenoids and saponins in significant amounts (Fontes et al., 2023). *Morinda citrifolia* L can lower blood sugar levels due to its antioxidant activity contained in Morinda citrifolia L in the form of phenolics and flavonoids. The vitamin C content of *Morinda citrifolia* L can inhibit the formation of superoxide radicals, hydroxyl radicals, peroxyl radicals, singlet oxygen and hydrogen peroxide. Vitamin C is one of the antioxidants which is a chemical compound that can prevent oxidation reactions. Ascorbic acid contained in the ethanol extract of *Morinda citrifolia* L can lower sorbitol levels and reduce protein glycation so that blood sugar levels will decrease (Algenstaedt et al., 2018; Srinivasahan & Durairaj, 2014).

As the days go by, insulin in mice will experience decreased sensitivity and eventually experience insulin resistance. When insulin can no longer regulate glucose, there will be a decrease in glucose transporters, especially GLUT-4, because GLUT-4 is the only GLUT that binds to insulin, and is present in the plasma membrane when insulin is present. Day by day, glucose metabolism in the body will become increasingly uncontrolled until the sufferer is diagnosed with diabetes mellitus (Wasik & Lehtonen, 2018). Giving insulin leaf extract and noni fruit extract to mice induced by alloxan can reduce blood sugar levels due to the content of secondary metabolites. Several secondary metabolites that are known to have anti-hyperglycemic activity include flavonoids, alkaloids, and tannins (Haryanto et al., 2023; Rusli, 2022) . Based on research Pakaya et al. (2024) and Sogandi (2019)), insulin leaves and noni fruit are known to have these three secondary metabolites.

Flavonoids are secondary metabolites that have a polyphenol structure with antioxidant activity. Widowati et al. (2021) stated that both metabolites have anti-hyperglycemic activity by increasing insulin production in pancreatic beta cells. This increase in production causes more glucose in the blood to enter the cells to be used as energy (Ghorbani, 2017). Alkaloids have been shown to have the ability to regenerate damaged pancreatic β cells (Arjadi et al., 2017). Antioxidant activity is able to capture free radicals which cause repair of pancreatic β cell damage that causes DM 1 (Wang & Wang, 2017). With the improvement of pancreatic tissue, there is an increase in the amount of insulin in the body so that blood glucose will enter the cells so that there is a decrease in blood sugar in the body

Tannins are known to stimulate glucose and fat metabolism so that the accumulation of these two sources of calories in the blood can be avoided. Tannins also have hypoglycemic activity, namely by increasing glycogenesis. Tannins can stimulate the regeneration of pancreatic beta cells, which are responsible for insulin production. By increasing the number of these cells, tannins help increase insulin secretion, which in turn lowers blood glucose levels. Tannins can inhibit glucose absorption in the small intestine, thereby reducing the amount of glucose that enters the bloodstream. This helps lower blood sugar levels after eating (Škorjanc, 2021). Tannins can also increase glucose transport by activating insulin-mediated signaling pathways, thereby increasing glucose absorption by body cells (Gerwen & Shun-shion, 2023). Tannins have strong antioxidant properties, which help protect body cells from free radical damage. This is especially important in the context of diabetes, where oxidative stress is often

increased. This compound also plays a role in increasing glycogenesis, which is the process of forming glycogen from glucose, which helps reduce blood glucose levels by preventing the accumulation of glucose in the blood (Omar et al., 2022).

The results of this study indicate that the increase in blood glucose levels of test animals after alloxan induction is related to pancreatic organ damage. In this study, several treatment groups that had been given insulin leaf extract, noni fruit extract and a combination of both extracts for 35 days experienced a decrease in blood sugar levels, especially in the combination treatment of insulin leaf extract at a dose of 150 mg/kgBW + noni fruit extract at a dose of 62.5 mg/kgBW was able to reduce blood sugar levels after alloxan induction when compared to the positive control group (P3). The effect of reducing blood sugar levels due to the administration of a combination of insulin leaf extract at a dose of 150 mg/kgBW + noni fruit extract at a dose of 62.5 mg/kgBW after alloxan induction was significant, which means it was the most effective in reducing blood sugar levels in mice. The interaction of the combination of active ingredients is very likely to occur. The combination between active ingredients can show a synergistic effect (potentiation), an additive effect or an antagonistic effect. If a combination that has a similar or dissimilar work is given, then the combination effect can be additive (double the effect), synergistic (greater than double), or antagonistic (the effect of one or both extracts decreases). A profitable combination is of course a combination that has a synergistic effect on the active ingredients. The combination of synergistic effects is the goal pursued in the development of medicinal plants so that the study of the synergistic effects between active ingredients is of particular concern. In this study, the combination of insulin leaf extract at a dose of 150 mg/kgBW and noni fruit extract at a dose of 62.5 mg/kgBW gave a synergistic effect and had an average blood sugar result of 83 mg/dl compared to other treatment groups.

Number of Spermatogenic Cells (Number of Spermatogonium, Number of Spermatocytes, Number of Spermatids)

Spermatogonium

The results of statistical analysis obtained data that there was an effect ($p < 0.05$) of administering insulin leaf extract (*Smallanthus sonchifolius*), noni fruit extract (*Morinda citrifolia* L) and a combination of both extracts at various doses on the number of spermatogonium in diabetic mice that induced alloxan. The results of the Duncan test are as shown in the Figure 2.

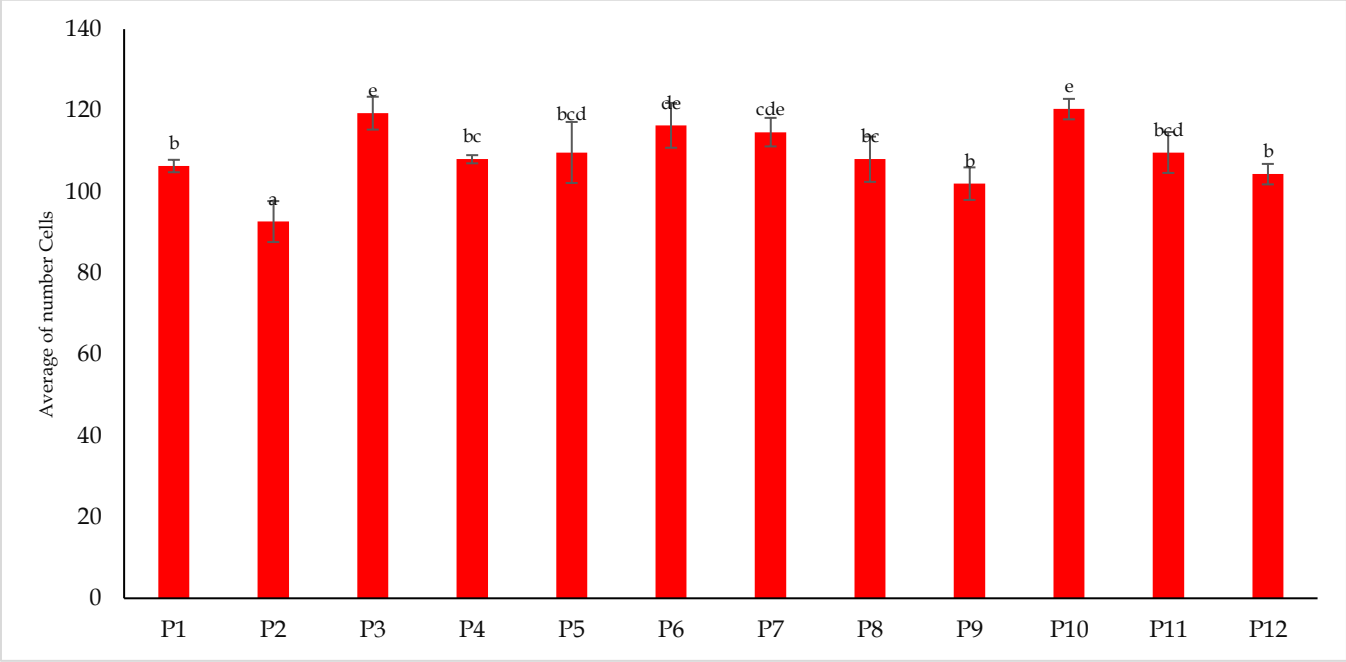

Figure 2. Average result of spermatogonia count the same letters are not significantly different: P1 control; P2 Negative control; P3 Positive control; P4 Insulin leaf extract 250mg/KgBW; P5 Insulin leaf extract 300mg/KgBW; P6 Insulin leaf extract 350mg/KgBW; P7 Noni fruit extract 100mg/KgBW; P8 Noni fruit extract 125mg/KgBW; P9 Noni fruit extract 150mg/KgBW; P10 Combination of insulin leaf extract 125 mg/kg BW + noni fruit extract 50 mg/KgBW; P11 Combination of insulin leaf extract 150 mg/kg BW + noni fruit extract 62.5mg/KgBW, and P12 Combination of insulin leaf extract 175 mg/kg BW + noni fruit extract 75mg/KgBW

Spermatocytes

The results of statistical analysis obtained data that there was an effect ($p < 0.05$) of administering insulin leaf extract (Smallanthus sonchifolius), noni fruit extract (Morinda citrifolia L) and a combination of both extracts at various doses on the number of spermatocytes in diabetic mice induced by alloxan. The results of the Duncan test are as shown in the Figure 3.

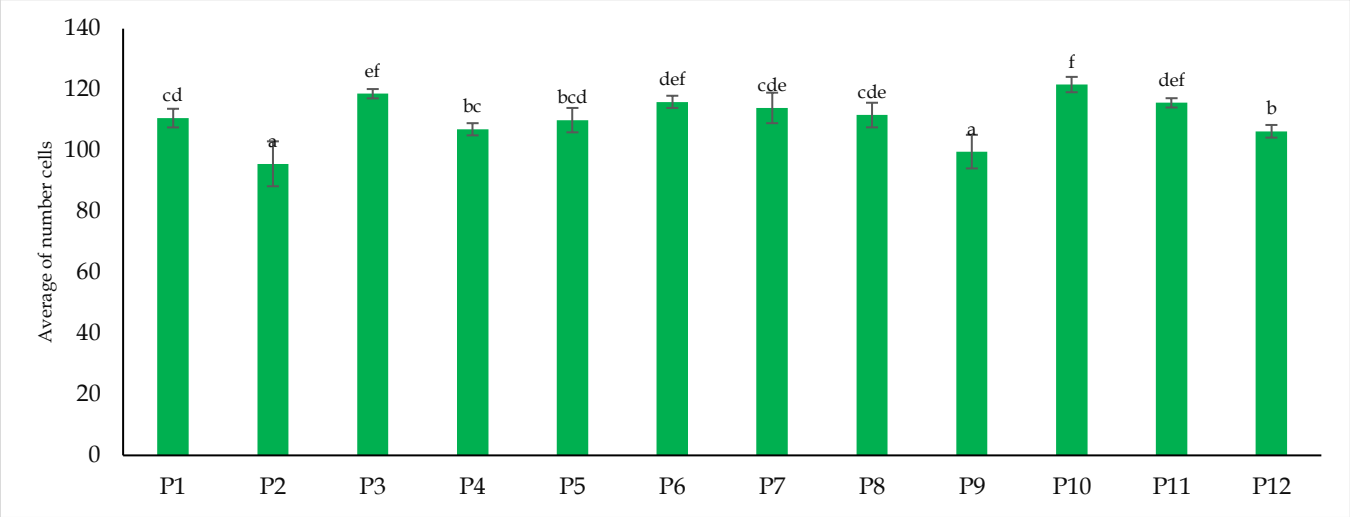

Figure 3. Average number of spermatocyte cells the same letters are not significantly different: P1 control; P2 Negative control; P3 Positive control; P4 Insulin leaf extract 250mg/KgBW; P5 Insulin leaf extract 300mg/KgBW; P6 Insulin leaf extract 350mg/KgBW; P7 Noni fruit extract 100mg/KgBW; P8 Noni fruit extract 125mg/KgBW; P9 Noni fruit extract 150mg/KgBW; P10 Combination of insulin leaf extract 125 mg/kg BW + noni fruit extract 50 mg/KgBW; P11 Combination of insulin leaf extract 150 mg/kg BW + noni fruit extract 62.5mg/KgBW, and P12 Combination of insulin leaf extract 175 mg/kg BW + noni fruit extract 75mg/KgBW.

Spermatids

The results of statistical analysis obtained data that there was an effect ($p < 0.05$) of administering insulin leaf extract (*Smallanthus sonchifolius*), noni fruit extract (Morinda citrifolia L) and a combination of both extracts at various doses on the number of spermatids in diabetic mice induced by alloxan. The results of the Duncan test are as shown in the Figure 4.

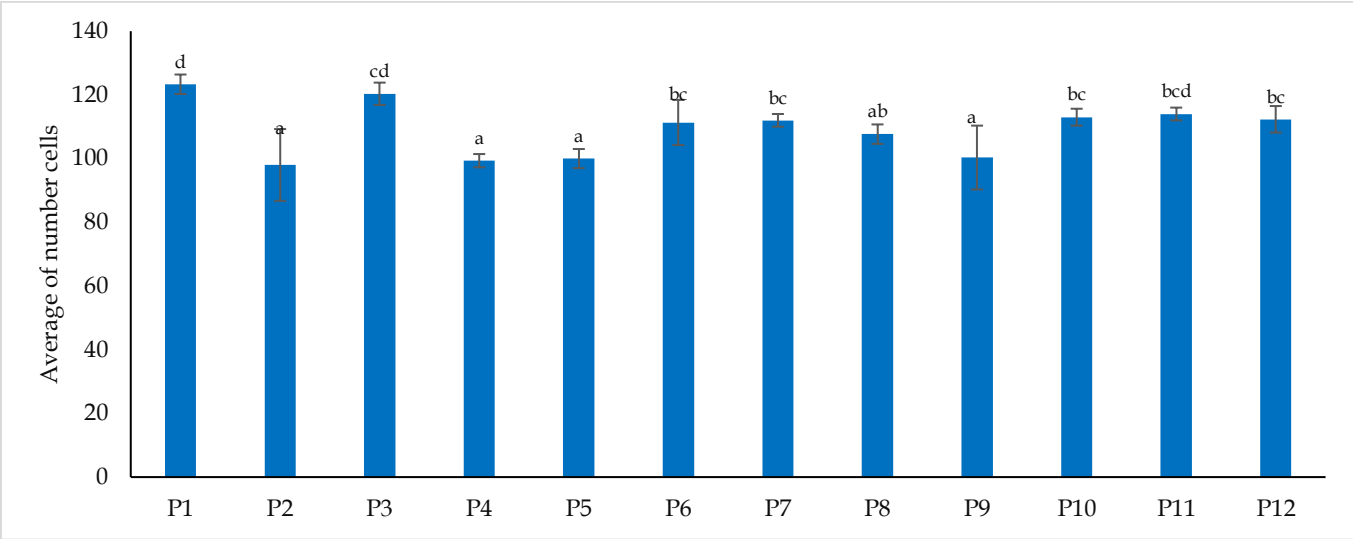

Figure 4. Average results of spermatid cell count the same letters are not significantly different: P1 control; P2 Negative control; P3 Positive control; P4 Insulin leaf extract 250mg/KgBW; P5 Insulin leaf extract 300mg/KgBW; P6 Insulin leaf extract 350mg/KgBW; P7 Noni fruit extract 100mg/KgBW; P8 Noni fruit extract 125mg/KgBW; P9 Noni fruit extract 150mg/KgBW; P10 Combination of insulin leaf extract 125 mg/kg BW + noni fruit extract 50 mg/KgBW; P11 Combination of insulin leaf extract 150 mg/kg BW + noni fruit extract 62.5mg/KgBW, and P12 Combination of insulin leaf extract 175 mg/kg BW + noni fruit extract 75mg/KgBW.

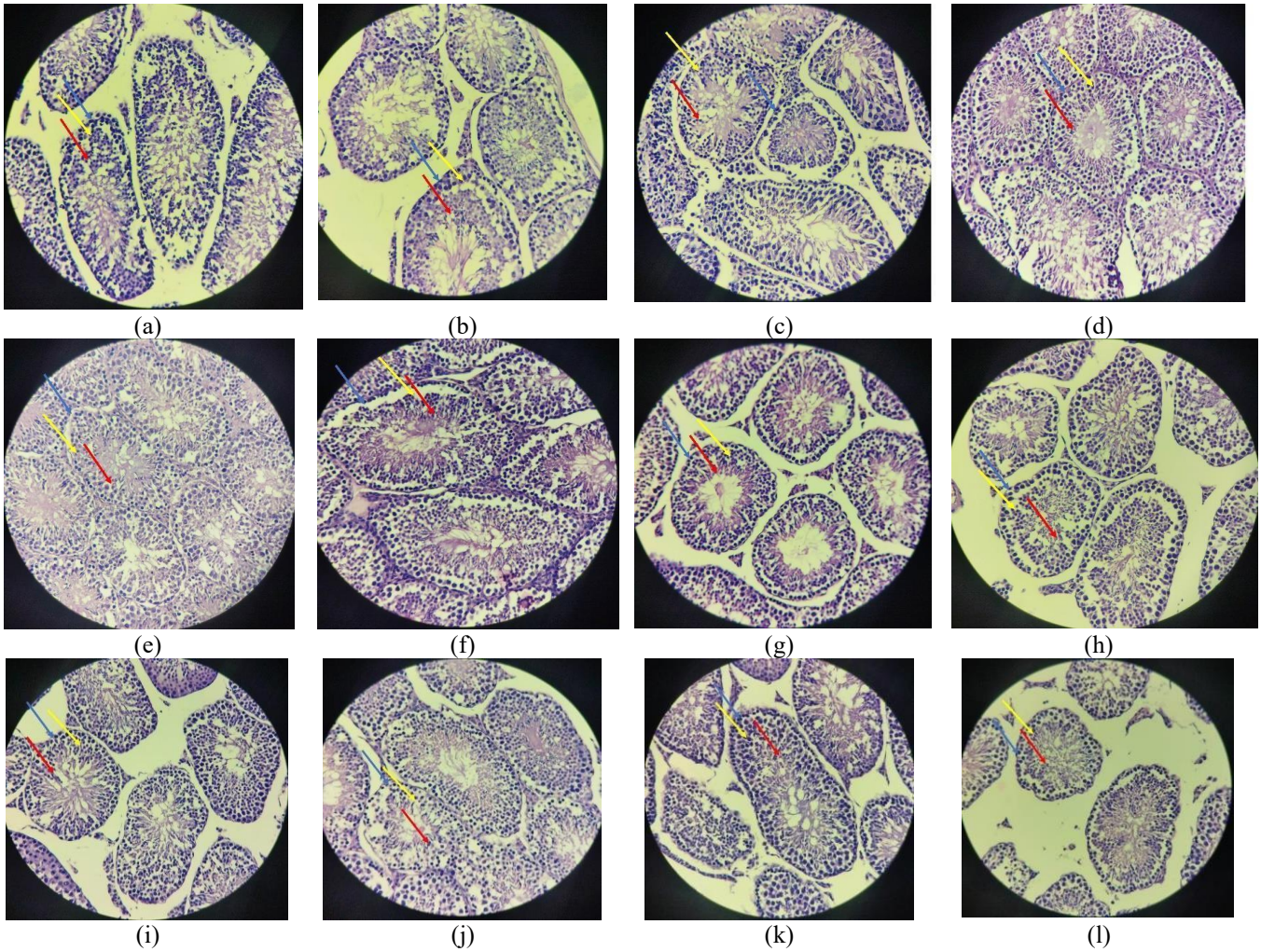

Figure 5. Histological visualization of seminiferous tubules Spermatid cells (red), Spermatocyte cells (blue), Spermatogonium cells (yellow): (a) P1 control; (b) P2 Negative control (c) P3 Positive control; (d) P4 Insulin leaf extract 250mg/KgBW; (e) P5 Insulin leaf extract 300mg/KgBW; (f) P6 Insulin leaf extract 350mg/KgBW; (g) P7 Noni fruit extract 100mg/KgBW; (h) P8 Noni fruit extract 125mg/KgBW; (i) P9 Noni fruit extract 150mg/KgBW; (j) P10 Combination of insulin leaf extract 125 mg/kg BW + noni fruit extract 50 mg/KgBW; (k) P11. Combination of insulin leaf extract 150 mg/kg BW + noni fruit extract 62.5mg/KgBW; and (l) P12 Combination of insulin leaf extract 175 mg/kg BW + noni fruit extract 75mg/KgBW

The testes are organs that are susceptible to free radicals so that the presence and high levels of these compounds disrupt spermatogenesis and the integrity of spermatogenic cell membranes. Spermatogenic cells have membranes containing polyunsaturated fatty acids. In diabetic mice, there is a decrease in spermatogenesis and apoptosis of spermatogenic cells in the epithelium of the seminiferous tubules of the testes. Spermatogenesis can be disrupted due to microangiopathy in hyperglycemic conditions which can disrupt the provision of nutrients to the epithelium of the seminiferous tubules and apoptosis of spermatogenic cells. Apoptosis of spermatogenic cells occurs due to increased ROS in the testes (Zhou et al., 2025). The meeting of spermatogenic cells with free radicals will cause a lipid peroxidation reaction. Disruption of membrane integrity, increased membrane fluidity, and inactivation of membrane bonds with enzymes and receptors due to lipid peroxidation reactions. Spermatogenesis in the testes is also influenced by the presence of Leydig cells that secrete testosterone for use in the seminiferous tubules. In spermatogenesis, testosterone is needed by germ cells to divide to form spermatozoa, especially when forming secondary spermatocytes at the meiosis division stage (Grande et al., 2022). If the number of Leydig cells decreases, the spermatogenesis process will also be inhibited. This condition causes abnormalities in spermatogenic cells so that the number of sperm formed decreases (Zirkin & Papadopoulos, 2018). In this study, diabetic mice in the negative control treatment (P2) had a thinner seminiferous tubule epithelial layer due to decreased spermatogenesis and apoptosis of spermatogenic cells. Apoptosis caused by ROS can also cause a decrease in the number of Leydig cells and loss

of connective tissue in the interstitial space (Monageng et al., 2023).

In insulin leaves and noni fruit there are saponin compounds. Saponins can be triterpenoid glycosides and steroids. The effects of saponins can interfere with membrane permeability and antifertility. Saponins can form pores in the membrane so that they can enter cells and inhibit genes responsible for steroidogenesis (Zheng & Gallot, 2020). In this case, saponins have cytotoxic properties so that they can reduce the number of spermatogenic cells (Timar et al., 2024). This symptom can be seen in the treatment of 150 mg/KgBW noni fruit extract which produces the least number of spermatogenic cells compared to other extract treatment groups.

Insulin leaf extract, noni fruit extract and a combination of both extracts can increase spermatogenic production. This increase can be caused because both extracts contain flavonoids that can reduce ROS in testicular tissue (Mohammadi et al., 2023). Flavonoids have a similar structure to cholesterol and other steroids, so this compound can affect androgen production in Leydig cells. Flavonoids can inhibit the apoptosis process in germ cells of diabetic mice testes and also have a cytoprotective effect on testicular cells thereby increasing spermatogenesis (Mohammadi et al., 2023). Myricetin is one type of flavonoid found in insulin leaf extract and noni fruit extract. Based on a study by Cormier et al. (2017) myricetin induction in diabetic mice can increase cAMP-dependent expression of StAR, Cyp11a1, and Fdx1, which contributes to increased steroidogenesis in Leydig cells. Myricetin is very effective in eliminating various ROS and executing anti-oxidative activities due to the large number of active hydroxyl groups. Myricetin can reduce the increase in free radical production during cell swelling and ischemic injury (Nadalin et al., 2023). Based on a study by Aquila et al., (2012), myricetin at low concentrations can increase sperm motility and viability. Low levels of ROS are essential for normal sperm function such as capacitation, hyperactivation, and acrosome reaction. These processes are critical for successful fertilization. Specifically, ROS activate adenylyl cyclase, which causes increased levels of cyclic AMP (cAMP), which phosphorylates proteins required for these functions. ROS facilitate capacitation by increasing sperm motility and preparing sperm for fusion with the oocyte. ROS also play a role in lipid peroxidation, which can alter membrane characteristics that are important for sperm function. For example, low concentrations of hydrogen peroxide can stimulate capacitation and the acrosome reaction (Castleton et al., 2022; Wang et al., 2025). However, when ROS levels exceed physiological thresholds, this can cause oxidative stress, resulting in significant damage to sperm membranes, DNA, and overall sperm viability. High ROS levels are associated with lipid peroxidation, which disrupts membrane integrity and impairs motility (Aitken, 2017)). ROS are useful for the complex process of male germ-cell proliferation and maturation, from diploid spermatogonia through meiosis to mature haploid spermatozoa (Guerriero et al., 2014).

In the study, diabetic mice (negative control) had a lower number of spermatogonia, spermatocytes and spermatids compared to the control or positive control and other treatments. The decrease in the number of spermatogenic cells is thought to be due to an increase in excessive metabolism and causes an increase in the production of free radicals, causing spermatogenic cells to become unstable (Nurkarimah et al., 2017). The efficiency of spermatogenesis depends on the proliferation of spermatogonia and the loss of germ cells during meiosis and spermiogenesis. Diabetic mice (negative control) may have increased levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), indicating a disturbance in the hypothalamic-pituitary-gonadal axis. This hormonal imbalance is associated with decreased testosterone levels, which are very important for spermatogenesis and overall reproductive function. In addition, diabetes also causes Spermatogenesis Disorders. Previous studies have shown that diabetes causes decreased testicular volume, semen volume, sperm count, and sperm motility. In diabetic conditions, the number of spermatogenic cells decreases, and the structural integrity of the seminiferous tubules is disrupted. This disorder is associated with increased apoptosis of testicular cells, which further reduces sperm production (Long et al., 2018). Hyperglycemia also causes oxidative stress, which is characterized by an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses. Excessive ROS can damage cellular structures, including DNA and proteins in testicular cells, leading to impaired spermatogenesis. In addition, histological changes occur, namely in Histological studies in diabetic models have revealed significant degeneration of testicular tissue, including thickening of the basement membrane and loss of spermatogenic cells. These changes contribute to infertility by disrupting the normal spermatogenic environment (Maresch et al., 2018). The spermatozoa membrane has unsaturated fatty acids so it is very suitable for free radicals. Lipid peroxidation in the spermatozoa membrane is one of the impacts that occurs due to ROS, which can cause increased membrane permeability and disruption of the respiratory chain and ATP production (Bibov et al., 2018).

The results of this study indicate that in the control treatment (P1), positive control (P3), and 9 treatment treatments, namely treatment using a dose of insulin leaf extract 250 mg/Kg BW, insulin leaf extract 300 mg/Kg BW, insulin leaf extract 350 mg/Kg BW, noni fruit extract 100 mg/Kg BW, noni fruit extract 125 mg/Kg BB, noni fruit extract 150 mg/Kg BW, a combination of insulin leaf extract 125 mg/Kg BW + noni fruit extract 50 mg/Kg BW, a combination of insulin leaf extract 150 mg/Kg BW + noni fruit extract 62.5 mg/Kg BW and a combination of insulin leaf extract 175 mg/Kg BW + noni fruit extract 75 mg/Kg BW, the spermatogenic layer in the seminiferous tubules is seen to be thicker and denser due to the growth of spermatocytes. According to Akhtar et al. (2020), the more active the spermatogenesis process that occurs, the more dense the lumen of the seminiferous tubules will appear. From these results, it can be said that insulin leaf extract and noni fruit extract along with both combinations given to diabetic mice have an effect on increasing spermatogenesis. In this case, there was an increase in the number of spermatogonium cells, spermatocytes, and spermatids with insulin leaf extract and noni fruit extract treatments, especially in the group of mice with a combination treatment of 125 mg/KgBW insulin leaf extract and 50 mg/KgBW noni fruit extract. Research conducted by Sukarjati et al. (2024) found that combination of Gynura precumbens and Swietenia mahagony Jacq extracts at a dose of 150 mg/Kg BW: 150 mg/Kg BW can reduce blood sugar levels and improve the quality of sperm and spermatogenic cells in hyperglycemic mice. The combination of pumpkin flour (*Cucurbita moschata*) and porang flour (*Amorphophallus muelleri Blume*) can reduce blood sugar levels and increase spermatozoa motility in alloxan-induced diabetic mice (Sukarjati et al., 2023).

The statistical data results also showed that the group of mice treated with insulin leaves 250 mg/Kg BW dose was too low to increase the number of spermatogenic cells shown by the results not significantly different from the control group (P1), while the group of mice treated with noni fruit extract 150 mg/Kg BW was too high, this was indicated by the results of the low number of spermatogenic cells from other treatment groups of mice. According to Mazidda et al., (2022), the high concentration of extract reduces effectiveness such as decreased motility and viability. So that the group of mice treated with a combination of insulin leaf extract 125 + noni fruit extract 50 had the most optimum effect on increasing the number of spermatogenic cells, although the number of spermatids was still lower than in the control group of mice (P1).

Conclusion

Administration of insulin leaf extract (*Smallanthus sonchifolius*), noni fruit extract (*Morinda citrifolia L*) and a combination of both extracts have an effect on reducing blood sugar levels in diabetic mice (*Mus muscullus*). And administration of a combination of insulin leaf extract (*Smallanthus sonchifolius*) at a dose of 150 mg/KgBW/day + noni fruit extract (*Morinda citrifolia* L) at a dose of 62.5 mg/KgBW/day has the best effectiveness in reducing blood sugar levels in diabetic mice (*Mus muscullus*) compared to other treatment groups. Administration of insulin leaf extract (*Smallanthus sonchifolius*), noni fruit extract (*Morinda citrifolia* L) has an effect on increasing the number of Spermatogenic cells (Spermatogonium, Spermatocyte, Spermatids) in diabetic mice (*Mus muscullus*). And the administration of a combination of insulin leaf extract (*Smallanthus sonchifolius*) at a dose of 125 mg/KgBW/day + noni fruit extract (*Morinda citrifolia* L) at a dose of 50 mg/KgBW/day has the best effectiveness in increasing the number of spermatogenic cells (Spermatogonium, Spermatocyte, Spermatids) in diabetic mice (*Mus muscullus*) compared to other treatment groups.

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