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Enhancing Spermatozoa Quality and Spermatogenic Cell in Hyperglycemic Mice (*Mus musculus L.*) with Sambung Nyawa (*Gynura procumbens*) and Mahagany (*Swietenia mahagoni* jacq) extracts, Alone and in Combination

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Abstract: This study aimed to investigate the effect of the ethanol extract of sambung nyawa (*Gynura procumbens*) leaves, mahogany (*Swietenia mahagoni* jacq) seeds, and their combination on the motility, viability, morphology, and concentration of spermatozoa, spermatogonia, spermatocytes, and spermatids in hyperglycemic mice induced with 10% dextrose. A total of 48 male mice were used as experimental animals, and the extracts were administered orally for 35 days. On day 36, spermatozoa and testicular tissues were collected for analysis. The results showed that the extracts at various doses had the potential to reduce blood glucose levels and improve the motility, viability, morphology, and concentration of spermatozoa and germ cells in hyperglycemic mice. Result:The combination of sambung nyawa leaves and mahogany seeds at a dose of 150:150 mg/kg BW was found to be the most effective in reducing blood sugar levels and improving sperm parameters. Therefore, these extracts have the potential to be used as alternative herbal ingredients with similar effects to metformin in reducing diabetes and improving fertility.

Keywords: Dabetes mellitus *Gynura procumbens* leaves; mahagany (*Swietenia mahagoni jacq*); *Mus musculus L.* sperm quality; Spermatogenic cells

Introduction

One of the chronic diseases which is the most important public health problem that still cannot be resolved is Diabetes Mellitus (DM). Every year DM sufferers have increased. Indonesia ranks seventh in the list of 10 countries with the largest number of DM sufferers in the world (Harista & Lisiswanti, 2017). DM is defined as a disease or chronic metabolic disorder characterized by high blood glucose levels accompanied by impaired carbohydrate, lipid and protein metabolism due to impaired insulin function. Impaired insulin function can be caused by a lack of response of body cells to insulin (Putri & Tjitraresmi, 2018).

DM may cause impotence, ejaculation disorders, and damage spermatogenesis due to the increase in Reactive Oxygen Species (ROS) which can damage mitochondrial membranes, thereby inducing apoptosis of spermatozoa cells (Condorelli et al., 2018). Increased ROS in the body can damage enzymes that function as antioxidants and free radicals, causing oxidative stress. Oxidative stress acts as a mediator of damage to the plasma membrane, thereby reducing the function of spermatozoa. In addition, the damage to blood vessel endothelium may cause microangiopathy, which causes disruption of nutrient provision through the blood vessels to spermatozoa-forming tissues.

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DM patients usually use pharmacological therapy consisting of oral hypoglycemic drugs, insulin injections, and other antidiabetic injections. The disadvantages of these drugs are that they are not cost effective and have side effects. Therefore, most people have started to turn to alternative medicine by using plants. Thus, a study on the potential of Indonesian medicinal plants that have antidiabetic activity without causing side effects of hypoglycemia, which at the same time can improve the quality of spermatozoa, needs to be done. One of the plants that is often used by the community to treat DM is the *Sambung nyawa* (*Gynura procumbens*) plant. This plant contains chemical compounds such as flavonoids, tannins, saponins, steroids, polyphenol compounds, essential oils and sterols (Timotius & Rahayu, 2020). According Jobaer et al. (2023) *Gynura procumbens* contains phytol, lupeol, stigmasterol, friedelanol acetate, amyrin, and a mixture of stigmasterol and sitosterol. *Gynura procumbens* as antidiabetic.

Research conducted by Kamaruzaman et al. (2018) used ethanol extract of *sambung nyawa* leaves to treat DM and infertility in mice by observing testicular histology and androgen hormone levels. The doses used were 150, 175, and 250 mg/kg BW, while the positive control was given with 65 mg/kg BW metformin. The experimental animals were 42 white rats induced by streptozotocin as much as 50 mg/kg BW orally. The results showed that administration of *sambung nyawa* leaves ethanol extract at a dose of 250 mg/kg BW was significantly able to treat infertility in diabetic male mice through upregulation of proteins associated with spermatozoa maturation.

Another herbal plant that has antidiabetic activity is the mahogany (*Swietenia mahagoni jacq*) seeds. Mahogany seeds contain saponins, flavonoids, and alkaloids as well as swietenin which function as hypoglycemic agents (Ahmad et al., 2019). According Asmara (2018), ethanolic extract of *Swietenia mahagony* seed contains tetranortriterpenoid or limonoid group. Study conducted by Fiorenza et al. (2022) used 25 male rat (*Rattus norvegicus)* that were conditioned to be diabetic using alloxan, which were then given with mahogany seed extract at a combined doses of 250, 500, and 1000 mg/kg BW for 14 days. The results showed a significant decrease in blood glucose levels at a dose of 500 mg/kg BW (Fiorenza et al., 2022). Consumption of mahogany seeds can be used as a complementary therapy in nursing to reduce blood sugar (Ardiansyah et al., 2021).

Mahagany seed tea (*Swietenia mahagoni*) has an effect on reducing MDA levels in type 2 diabetes mellitus rats induced by alloxan, with a dose of 15 grams/kg BW which is the optimal dose in reducing glucose levels and MDA levels (Auliah & Asri, 2019). *Swietenia mahogany* contain several classes of active compounds were identified qualitatively in the extract, which are flavonoids, phenolics, terpenoids, steroids, glycosides, and saponins. Mahogany seed extract contains alkaloids, flavonoids, saponins and triterpenoids. 96% ethanol extract and water extract of mahogany seeds have potential as antidiabetic compounds based on their ability to inhibit α-glucosidase in vitro (Nurcholis et al., 2019). That 70% ethanol extract of mahogany seed has a characterize extract that meets the standards and contains several active phytochemical compounds as potential antidiabetic agents (Gede et al., 2022). According Sinurat & Budi (2023) reported that giving dry extract of mahogany seeds (*Swietenia mahogani Jacq*) was effective in reducing blood sugar levels in male Wistar rats given a high fructose diet. Yudhani et al. (2021) reported *Swietenia macrophylla* seeds as antidiabetic candidate is supported by many studies that have documented their non-toxic and hypoglycemic effects (Yudhani et al., 2021). However, data on the combined extract of *sambung nyawa* leaves and mahogany seeds which function to reduce blood sugar levels and improve sperm quality and spermatogenic cells in hyperglycemic mice have not been reported.

The purpose of this study was to examine the effect of the provision of *sambung nyawa* (*Gynura procumbens*) leaves ethanol extract, mahogany (*Swietenia mahagoni jacq*) seeds and the combination of both extracts in various doses on increasing motility, viability, morphology and concentration of spermatozoa, spermatogonia, spermatocytes and spermatids of hyperglycemic mice (*Mus musculus L.*). The subjects of this study were mice (*Mus musculus L.*) induced by 10% dextrose. Dextrose is a sugar in a simple chemical structure with the same chemical formula as glucose. If given in excess, dextrose can cause hyperglycemia, which is characterized by the destruction of pancreatic beta cells and the inhibition of insulin synthesis and secretion. This research is important for developing herbal medicine for DM and improving the quality of spermatozoa. Research on the potential for sambung nyawa (*Gynura procumbens*), mahogany seeds (*Swietenia mahagoni jacq*) and their combination to reduce blood glucose levels and improve the quality of hyperglycemic mouse spermatozoa (*Mus musculus* L.) has never been carried out.

Method

This research was an experimental study using completely randomized design. The experimental animals used were male mice (*Mus musculus L.*) aged 2 months weighing 27-30 grams, obtained from Pusvetma

Surabaya, Indonesia, a total of 48 mice. In this study there were twelve treatments, each of which was repeated four times.

Extract production

Sambung nyawa leaves were air-dried to become simplicia. Mahogany seeds were dried as well. The simplicia of *sambung nyawa* leaves and mahogany seeds were each mashed using a blender. The powder of *sambung nyawa* leaves and mahogany seeds, each of 100 grams, was wrapped in cloth then tied and each soaked in 1000 ml of 80% ethanol in a jar for three days. Stirring was done every day. After three days, each filtrate was concentrated using a rotary evaporator.

Experimental animals preparation

Two months old male mice weighing 27-30 grams totaling 48 mice were acclimatized for seven days. The mice were given with standard feed and given access to water ad libitum. The mice were fasted for eight hours prior to testing the initial blood glucose level and weighed to calculate the treatment dose.

Extract treatment

To create a state of hyperglycemia in the experimental animals, after being adapted for seven days, all treatments except the control group (non-diabetics) were induced with 10% dextrose for seven days which was given as a drink. Then, the blood glucose levels of the mice were examined on day eight after induction. If the blood glucose level was > 150 mg/dl, the mice were declared as diabetic (Kemila, 2009., Hardiyani, 2013). These mice were used in the next stage of the research, namely administration of metformin, *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and the combination of both extracts.

The mice were divided into six groups, group A: control (non-diabetic), B. Diabetic, C. Metformin, D. *Sambung nyawa* leaves extract, E. Mahogany seed extract, and F. A combination of *sambung nyawa* leaves extract and mahogany seeds extract. The dose used was metformin 112 mg/kg BW, control (without treatment), Groups D and E were each divided into three parts: doses D1,E1: 200 mg/kg BW, D2,E2: 250 mg/kg BW, and D3.E3: 300 mg/kg BW. Group F was divided into three parts with doses of 100:100 mg/kg BW, 125:125 mg/kg BW, and 150:150 mg/kg BW.

The extract was given orally using a sonde with a volume not exceeding the mice intragestic (0.5 ml). The extract was given to the mice once a day for 35 days at a dose that was calculated according to mg/kg body weight of the mice. On day 36, all mice were anesthetized with ether, dissected and the testes were isolated to make histological preparations, and the spermatozoa were collected from the cauda epididymis. The sperm was stained with Eosin Y to observe viability, sperm were stained with Safranin-Crystal Violet to observe morphology. Testicular histology preparations were stained according to laboratory standards.

Observation

Observation of blood glucose levels

Blood glucose levels were measured three times, after adaptation (healthy mice), on the seventh day after being induced with 10% dextrose for 7 days to determine if the mice were diabetic or not, and on the 36th day after the administration of the extract and metformin.

Observation of spermatozoa quality and testicular histology

Spermatozoa in the cauda were removed and placed in a watch glass which was then treated with Sperm Washing Media, then observed for sperm motility, viability, morphology and concentration. Motility was calculated on 100 sperm with the criteria of sperm moving fast straight forward, sperm moving slowly straight forward, sperm moving in place, and sperm not moving (dead). Viability was calculated based on 100 red colored and transparent sperm, morphology was calculated based on sperm normality and abnormality, while concentration was calculated using a Neubauer haemocytometer. In testicular histology preparations, the observations were made by counting the number of spermatogonia, spermatocytes and spermatids.

Data analysis

Data obtained were then analyzed using the One-Way Anova test with a confidence level ($p < 0.05$) to find out whether each treatment given had an effect on reducing blood sugar levels and increasing motility, viability, morphology, concentration of spermatozoa, spermatogonia, spermatocytes and spermatids. If the results were significant, the test was continued with the LSD (Least Significance Different) test to find out the differences in each treatment.

Result and Discussion

The increase in blood glucose levels after 10% dextrose was induced for 7 days. T test was conducted to determine the presence of differences in initial glucose levels with glucose levels after being given treatment with the results that initial blood glucose levels were significantly different ($p < 0.05$) from diabetic glucose levels. This showed that after 10% dextrose was induced, healthy mice would experience an increase in blood glucose levels. After statistical analysis using Anova, the

blood glucose level data from the treatment showed $p < 0.05$, which means that the extract of *sambung nyawa* (*Gynura procumbens*) leaves, the extract of mahogany seeds (*Swietenia mahagoni jacq*) and the combination of both extracts had a significant effect on reducing blood glucose levels in mice.

Furthermore, the LSD test showed result as in Table 1 and a decrease in blood sugar levels as shown in Figure 2.

Tabel 1. Initial blood glucose levels (healthy), after 10% dextrose induction (diabetic) and after treatment.

Treatment	Blood glucose levels in Mice (mg/dl)		
	Initial	Diabetic	after treatment
Diabet	128.75	192.00	148.25
Metformin	128.00	188.25	134.75
Control	117.75	158.50	131.75
SNA 200mg/Kg BW	119.50	167.75	142.50
SNB 250mg/Kg BW	122.75	196.75	137.00
SNC 300mg/Kg BW	127.50	203.50	135.50
BMA 200mg/Kg BW	120.25	167.00	141.75
BMB 250mg/Kg BW	131.00	208.25	139.50
BMC 300mg/KgBW	128.00	180.00	136.25
CA 100:100 mg/KgBW	129.00	180.00	138.00
CB 125:125 mg/KgBW	134.25	197.50	134.25
CC 150:150 mg/KgBW	122.00	206.00	130.00

Notes:
Diabet : Mice were induced by 10% dextrose without extract
SNA 200 : Diabetic mice received *sambung nyawa* leaves extract at a dose of 200 mg/kg BW
SNB 250 : Diabetic mice received *sambung nyawa* leaves extract at a dose of 250 mg/kg BW
SNC 300 : Diabetic mice received *sambung nyawa* leaves extract at a dose of 300 mg/kg BW
BMA 200 : Diabetic mice received mahogany seed extract at a dose of 200 mg/kg BW
BMB 250 : Diabetic mice received mahogany seed extract at a dose of 250 mg/kg BW
BMC 300 : Diabetic mice received mahogany seed extract dose of 300 mg/kg BW
CA 100 :100 : Diabetic mice received a combination extract of *sambung nyawa* leaves : mahogany seeds at a dose of 100:100 mg/kg BW
CB 125 :125 : Diabetic mice received a combination extract of *sambung nyawa* leaves : mahogany seeds dose of 125:125 mg/kg BW
CC 150 :150 : Diabetic mice received a combination extract of *sambung nyawa* leaves : mahogany seeds at a dose of 150:150 mg/kg BW

Blood sugar levels of hyperglycemic mice after treatment

The blood glucose levels of control mice, diabetic mice receiving metformin, *Sambung nyawa* extract, mahogany seeds and their combination at various doses were significantly different from the blood glucose levels of diabetic mice induced by 10% dextrose as seen in Table 2.

The results of the study proved that the administration of *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and their combinations at various doses reduced blood glucose levels and increased the quality of spermatozoa of hyperglycemic mice (*Mus musculus L.*). This occurred due to the presence of secondary metabolites contained in the *sambung nyawa* leaves extract and the mahogany seeds. The secondary metabolites contained in the *sambung nyawa* leaves extract are flavonoids, tannins, saponins, essential oil steroids and sterols, while mahogany seeds contain saponins, flavonoids, and alkaloids, terpenoids, anthraquinones, volatile oils, and swietenin (Naveen et al., 2014).

Table 2.LSD test of blood glucose levels in mice

Treatments	Mean ± SD
Combination 150:150 mg/kg BW	130.00 ± 5.71[a]
Control 0 mg/kg BW	131.25 ± 2.98[a]
Metformin	132.50 ± 11.17[a]
Combination 125:125 mg/kg BW	134.25 ± 3.30[a]
Sambung nyawa 300 mg/kg BW	135.25 ± 7.59[a]
Combination 100:100 mg/kg BW	135.75 ± 5.90[a]
Mahogany seeds 300 mg/kg BW	136.25 ± 7.67[a]
Sambung nyawa 250 mg/kg BW	137.00 ± 8.36[a]
Mahogany seeds 250 mg/kg BW	137.5 ± 4.20[a]
Sambung nyawa 200 mg/kg BW	138.25 ± 3.30[a]
Mahogany seeds 200 mg/kg BW	138.75 ± 5.37[a]
Dextrose 10%	148.25 ± 11.17[b]

Note: Different letters in different rows indicate significant differences (p=0.05) between groups

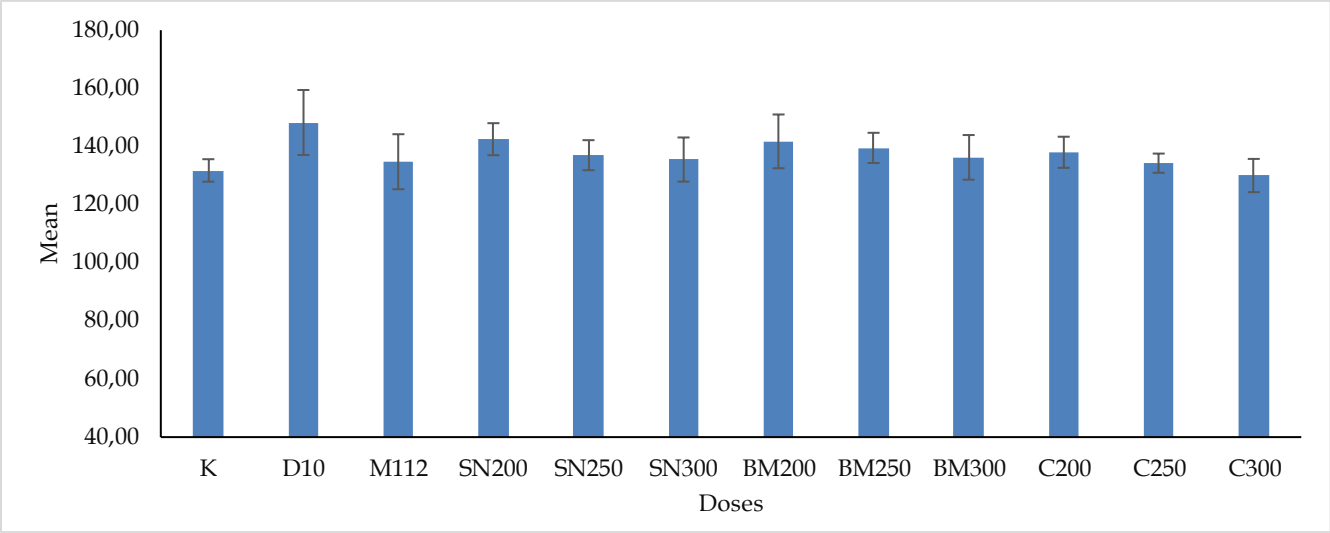

Figure 1. Comparison of the average reduction in blood glucose levels in mice

In this study, healthy mice were conditioned to become diabetic by induction with 10% dextrose for 7 days which was given as a substitute for drinking water. The mechanism of action of 10% dextrose is to damage pancreatic beta cells through the formation of free radicals and interfere with insulin secretion due to glucose stimulation through the inactivation of hexokinase. As a result, the pancreatic beta cells cannot secrete insulin, so that blood glucose levels increase.

Metformin lowers blood glucose levels by increasing the body's sensitivity to insulin produced by the pancreas through the activity of the AMP- activated protein kinase enzyme. It does not stimulate increased insulin production, and has no direct effect on pancreatic beta-cells. Improved pancreatic beta cells can resume their insulin secretory function, so that glucose levels are reduced. Metformin is a well-established antiperglycemic drug that can lower blood glucose levels in diabetic patients. Decreased blood glucose levels can also be caused by the presence of flavonoid, saponin, tannin and swietenin compounds. Flavonoid acts as antioxidant compound that suppresses free radicals in the body so that they can repair damaged pancreatic beta cells which can then increase insulin secretion (Donny Risnanda et al., 2020).

Saponin plays a role in pancreatic regeneration, causing an increase in the number of pancreatic beta cells and islets of Langerhans which will increase insulin secretion, so that the blood glucose levels will decrease (Astuti et al., 2017). Tannin can reduce free radicals by increasing the glucose digestion in the blood through insulin activity, resulting in the decrease of blood glucose (Al-Ishaq et al., 2019), while Swietenin acts by activating insulin responsive genes that can stimulate insulin to form and translocate GLUT (glucose transport) to the cell membranes in peripheral organs, so that peripheral glucose absorption and use increases, then blood glucose will enter the cells and the decrease of blood glucose finally occurs (Shiming et al., 2021).

Spermatozoa motility

ANOVA test showed that extract of *sambung nyawa* (*Gynura procumbens*) leaves, the extract of mahogany (*Swietenia mahagoni jacq*) seeds and their combination had a significant effect ($p<0.05$) on increasing spermatozoa motility in diabetic mice. Furthermore, the LSD test was carried out with results as shown in Table 3.

Table 3. LSD test on the motility of mice spermatozoa.

Types of extract	Mean ± SD
Diabetic 10% Dextrose	48.5 ± 3.1[a]
Mahogany seeds 200 mg/kg BW	61.5 ± 1.29[b]
Sambung nyawa 200 mg/kg BW	61.75 ± 2.5[b]
Mahogany seeds 250 mg/kg BW	62 ± 2.58[b]
Sambung nyawa 250 mg/kg BW	62.25 ± 2.21[b]
Mahogany seeds 300 mg/kg BW	62.75 ± 2.5[b]
Sambung nyawa 300 mg/kg BW	63.25 ± 2.5[b]
Combination 100 : 100 mg/kg BW	63.5 ± 3.1[b]
Combination 125 : 125 mg/kg BW	64.25 ± 4.34[b]
Metformin 112 mg/kg BW	65.25 ± 5.61[b]
Control 0 mg/kg BW	66 ± 4.96[b]
Combination 150 : 150 mg/kg BW	66.5 ± 4.04[b]

Note:Different letters in different rows indicate significant differences (p=0.05) between groups

Table 3 shows that the spermatozoa motility of mice induced by 10% dextrose was significantly different ($p<0.05$) from the spermatozoa motility of control mice, diabetic mice receiving metformin, *Sambung nyawa* extract, mahogany seeds and a combination of both extracts in various doses. The motility of mice spermatozoa decreased when induced by 10% dextrose, then increased or recovered after being treated with metformin, *sambung nyawa* (*Gynura procumbens*) leaves

extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and a combination of both extracts. In this study, the best increase in mice spermatozoa motility was obtained by administering a combination extract of *sambung nyawa* leaves and mahogany seeds at a dose of 150:150 mg/kg BW.

This study found that the sperm motility of diabetic mice induced by 10% dextrose was significantly different. The low motility of diabetic mice is because diabetes has an adverse effect on the specific activity of lactate dehydrogenase (LDH) in the testes. LDH enzymes are found in testicular germ cells, from primary spermatocytes to spermatozoa. LDH has a very vital role in sperm motility. LDH functions to catalyze the conversion of lactate to pyruvate in the Sertoli cell cytosol. The resulting pyruvate then enters the tricarboxylic acid cycle in the mitochondria. In the tricarboxylic acid cycle, the process of oxidative phosphorylation will produce ATP (Hakim et al., 2008). ATP is used in sperm motility. Thus, if LDH activity is disrupted, the ATP produced decreases, with the result of the decrease of sperm motility in the diabetic mice. After the administration of *sambung nyawa* leaves extract, mahogany seeds extract and their combinations, there was an increase in sperm motility. Possibly, the active substances in the extract were able to increase the LDH enzyme activity.

Glucose metabolism is of great important for sperm cells, either type 1 diabetes or type 2 diabetes could have detrimental effects on male fertility, especially on sperm quality, such as sperm motility, sperm DNA integrity, and ingredients of seminal plasma. Diabetes may influence the epigenetic modification during sperm spermatogenesis and that these epigenetic dysregulation may be inherited through the male germ line and passed onto more than one generation, which in turn may increase the risk of diabetes in offspring (Ding et al., 2015).

Spermatozoa Viability

ANOVA test showed that *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and the combination of both extracts in various doses had a significant effect ($p<0.05$) on increasing the viability of spermatozoa in diabetic mice and LSD analysis was carried out with the results as shown in table 4. Table 4 shows that the spermatozoa viability of mice induced by 10% dextrose was significantly different ($p<0.05$) from the spermatozoa viability of control mice, diabetic mice receiving metformin, *Sambung nyawa* extract, mahogany seeds and their combinations at various doses. Viability of mice spermatozoa decreased when induced by 10% dextrose, then increased after being treated with metformin, *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and their combinations. In this study the best increase in mice spermatozoa viability was reached by the administration of combined extract of *sambung nyawa* leaves and mahogany seeds at a dose of 150:150 mg/kg BW. The higher the dose of the extract given, the better the viability of the spermatozoa.

Table 4.LSD test on the viability of mice spermatozoa

Types of extract	Mean ± SD
Diabetic 10% Dextrose	62 ± 5.09[a]
Mahogany seeds 200 mg/kg BW	68.25 ± 4.64[b]
Sambung nyawa 200 mg/kg BW	68.5 ± 2.64[b]
Mahogany seeds 250 mg/kg BW	68.75 ± 2.98[b]
Sambung nyawa 250 mg/kg BW	69 ± 3.36[b]
Mahogany seeds 300 mg/kg BW	69.25 ± 3.5[b]
Combination 100:100 mg/kg BW	70.25 ± 4.64[b]
Metformin 112 mg/kg BW	71 ± 2.58[b]
Sambung nyawa 300 mg/kg BW	71.25 ± 4.03[b]
Combination 125: 125 mg/kg BW	71.75 ± 4.78[b]
Control 0 mg/kg BW	72.5 ± 5.44[b]
Combination 150: 150 mg/kg BW	73 ± 3.16[b]

Note: Different letters in different rows indicate significant differences ($p=0.05$) between groups

This study showed that sperm viability of diabetic mice induced by 10% dextrose was significantly different from that of control and treatment. Diabetic mice have high ROS. Sperm are vulnerable to attack by reactive oxygen species (ROS) due to their high unsaturated fatty acid content and lack of DNA repair mechanisms. ROS produce lipid peroxide from unsaturated fatty acids (PUFA/PolyUnsaturated Fatty Acid) which can be found in high amounts in sperm cell membrane phospholipids in the form of malondialdehyde (MDA) (Papadopoulou et al., 2022). High ROS results in high lipid peroxide which results in damage to the sperm plasma membrane (Dutta et al., 2019). This resulted in decreased sperm viability of diabetic mice. After the administration of *sambung nyawa* leaves extract, mahogany seeds extract and a combination of both substances, sperm viability increased because the active ingredients in the extract were able to ward off ROS.

Morphology

ANOVA test showed that *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and the combination of both extracts in various doses had a significant effect ($p<0.05$) on increasing the morphology of spermatozoa in diabetic mice and LSD analysis was carried out with the results as shown in table 5. The research results regarding the potential of *sambung nyawa* leaves (*Gynura procumbens*) extract, mahogany (*Swietenia mahagoni jacq*)

seeds extract and their combinations. The morphology of mice spermatozoa decreased when induced by 10% dextrose, then increased after being treated with metformin, *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and their combinations. In this study, the best improvement in the morphology of mice spermatozoa was achieved by administering a combination of *sambung nyawa* leaves extract and mahogany seeds extract at a dose of 150:150 mg/kg BW. The higher the dose of the extract given, the better the morphology of the spermatozoa.

Table 5. LSD test on the morphology of spermatozoa

Types of extract	Mean ± SD
Diabetic 10% Dextrose	63.25 ± 2.98[a]
Mahogany seeds 200 mg/kg BW	72 ± 3.65[b]
Sambung nyawa 200 mg/kg BW	72.5 ± 5.19[b]
Metformin 112 mg/kg BW	73.25 ± 4.03[b]
Sambung nyawa 250 mg/kg BW	73.25 ± 4.34[b]
Mahogany seeds 250 mg/kg BW	73.25 ± 5.05[b]
Mahogany seeds 300 mg/kg BW	74.5 ± 4.2[b]
Sambung nyawa 300 mg/kg BW	76.5 ± 5.8[b]
Combination 100 : 100 mg/kg BW	77 ± 3.91[b]
Combination 125 : 125 mg/kg BW	77.75 ± 4.57[b]
Control 0 mg/kg BW	78.5 ± 4.93[b]
Combination 150 : 150 mg/kg BW	78.5 ± 0.1[b]

Note: Different letters in different rows indicate significant differences (p=0.05) between groups

Table 5 shows that the spermatozoa morphology of mice induced with 10% dextrose was significantly different ($p<0.05$) from the spermatozoa morphology of control mice, diabetic mice receiving metformin, *Sambung nyawa* extract, mahogany seeds and their combinations at various doses. In this study, spermatozoa with normal morphology and abnormal morphology were found. Abnormal morphology caused by free radicals can cause chronic effects, resulting in impaired cell function, damage to cell structures and disturbances in the reproductive system (Mannucci et al., 2022). Abnormalities in mice spermatozoa can be identified, including small heads, irregular heads, hooks bent backwards, no hooks, broken sperm tails, short tailed sperm, crooked tails, and double tails. Spermatozoa abnormalities due to free radicals can occur during the process of spermatogenesis or can also occur after spermatozoa leave the seminiferous tubules. In this study, the increase in spermatozoa count with abnormal morphology in diabetic mice induced by 10% dextrose, compared to controls, may be due to oxidative stress on germ cell maturation and function in the mice. Increased oxidative stress and ROS production in diabetes often parallels with the increased expression of Cytochrome P-4502E1 isoform (CYP2E1). CYP2E1 generates reactive oxygen intermediates, such as superoxide radicals, which in turn can rapidly react with organic molecules to produce secondary free radicals and ROS. These conditions can change the antioxidant environment of the testes and epididymis, causing impaired germ cell development in the testes, with the result of the increase of sperm count with abnormal morphology. After the administration of *sambung nyawa* leaves extract, mahogany seeds extract and their combination, abnormal sperm counts in diabetic mice induced by 10% dextrose were not significantly different from controls. This may be because *sambung nyawa* leaves contain antioxidants (Kaewseejan et al., 2015) and mahogany seeds also contain antioxidants (Winata & Putri, 2019) which can provide protection against oxidative stress (Yaribeygi et al., 2020).

Concentration

ANOVA test showed that *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and the combination of both extracts in various doses had a significant effect ($p<0.05$) on increasing the concentration of spermatozoa in diabetic mice and LSD analysis was carried out with the results as shown in Table 6.

Table 6. LSD test on the concentration of spermatozoa.

Types of extract	Mean ± SD
Diabetic 10% Dextrose	73.75 ± 3.3[a]
Mahogany seeds 200 mg/kg BW	80.75 ± 3.3[b]
Sambung nyawa 200 mg/kg BW	81 ± 5.88[b]
Mahogany seeds 250 mg/kg BW	81.25 ± 4.42[b]
Sambung nyawa 250 mg/kg BW	82 ± 3.91[b]
Mahogany seeds 300 mg/kg BW	82.25 ± 7.84[b]
Sambung nyawa 300 mg/kg BW	83.25 ± 5.37[b]
Combination 100 : 100 mg/kg BW	83.75 ± 5.5[b]
Metformin 112 mg/kg BW	83.5 ± 4.69[b]
Combination 125 : 125 mg/kg BW	85 ± 3.55[b]
Control 0 mg/kg BW	85.5 ± 3.87[b]
Combination 150 : 150 mg/kg BW	89.75 ± 2.5[b]

Note: Different letters in different rows indicate significant differences (p=0.05) between groups

That the concentration of mice spermatozoa decreased when induced by 10% dextrose, then increased or recovered after being treated with metformin, *sambung nyawa* (*Gynura procumbens*) leaves extract, mahogany (*Swietenia mahagoni jacq*) seeds extract and their combinations. In this study, the best increase in the concentration of mice spermatozoa was achieved by administering combined extract of *sambung nyawa* leaves and mahogany seeds at a dose of 150:150 mg/kg BW. The higher the dose of the extract given, the better the concentration of spermatozoa. Table 6 shows that the spermatozoa concentration of mice induced by 10% dextrose was significantly different ($p<0.05$) from the spermatozoa of control mice, diabetic mice receiving

metformin, *Sambung nyawa* extract, mahogany seeds and their combinations at various doses.

Spermatozoa concentration is one of the important factors to support the success of fertilization. In this study, the sperm concentration of 10% dextrose-induced diabetic mice was significantly different from that of treatment and control mice. The low sperm concentration of diabetic mice may be due to the inhibition of spermatogenesis resulting from the high ROS in these mice. Furthermore, the administration of *sambung nyawa* leaves extract, mahogany seeds extract and their combination for 35 days could increase or restore sperm count of diabetic mice induced by 10% dextrose. The increase of spermatozoa count resulted from the presence of active compounds found in *sambung nyawa* leaves extract and mahogany seeds extract, one of which is essential oil. Essential oil acts as a very strong antioxidant compound providing a dominant protective effect from DNA damage caused by hydrogen peroxide H_2O_2.

In addition, essential oil acts as a free radical scavenger which then facilitates the delivery of cholesterol to be converted to pregnenolone by cytochrome P450cc enzymes in the mitochondrial membrane of the Leydig cells. Pregnenolone functions as a biosynthesis of testosterone hormone. The increase in pregnenolone produced by the Leydig cells will trigger an increase in testosterone production. Testosterone functions to control the process of spermatogenesis in meiosis division and in the process of spermiogenesis, so that it can launch the process of spermatogenesis. As a result, the concentration of spermatozoa produced will increase (Guardo et al., 2020).

Testosterone plays a very important role in improving the quality of spermatozoa, including increasing the concentration of spermatozoa. Stable free radical compounds will optimize the work of Sertoli cells and Leydig cells, so that the process of spermatogenesis returns to normal and the concentration of spermatozoa produced will increase (Asadi et al., 2017). Sterols will trigger an increase in active ingredients in the body, which will be followed by an increase in the hormone testosterone in the blood so that the process of spermatogenesis increases. This substance improves the activity of anterior pituitary cell membrane so there will be enhanced release of LH and FSH. The increase in LH and FSH will affect the testicular organs. Testicular membrane activity will be better so that the LH receptor affinity on Leydig cells will increase. LH will bind to receptors and will stimulate the formation and secretion of testosterone. FSH produced by the pituitary stimulates Sertoli cells to form sex hormone binding globulin (SHBG), which functions to bind testosterone produced by Leydig cells. This process will increase testosterone levels in the testes which is a requirement for the initiation of spermatogenesis process (Smith & Walker, 2014).

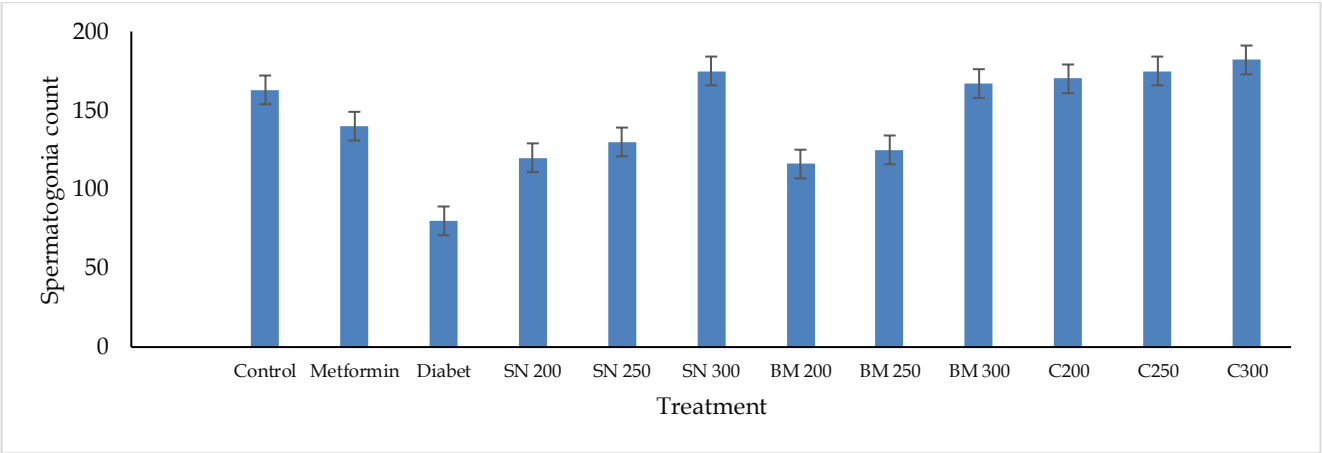

Figure 2. Comparison of the average number of spermatogonium cells from mice receiving *sambung nyawa* leaves extract, mahogany seeds extract and combined extracts

Spermatogonia

Figure 2 shows that mice induced with 10% dextrose had a low number of spermatogonia cells due to high sugar levels without being treated. Mice that received metformin had a higher number of spermatogonia cells compared to mice that received *sambung nyawa* leaves extract and mahogany seeds extract at a dose of 200 mg/kg BW and a dose of 250 mg/kg BW. The higher the dose of *sambung nyawa* leaves extract and mahogany seeds extract and the combination of both, the higher the number of spermatogonia. The highest increase in the number of spermatogonium cells occurred in the administration of a combined extract of *sambung nyawa* leaves and mahogany seeds at a dose of 150:150 mg/kg BW.

Spermatocytes

Figure 3 shows that mice induced with 10% dextrose had a low number of spermatocytes due to high sugar levels without being treated. Mice that received metformin had a higher number of spermatocytes compared to mice receiving *sambung nyawa* leaves extract, mahogany seeds extract, and combined extracts at a dose of 200 mg/kg BW and a dose of 250 mg/kg BW. The higher the dose of *sambung nyawa* leaves extract and mahogany seeds extract and the combination of both, the higher the number of spermatocyte cells. The highest increase in the number of spermatocytes occurred in the administration of combined extract of *sambung nyawa* leaves and mahogany seeds at a dose of 150:150 mg/kg BW.

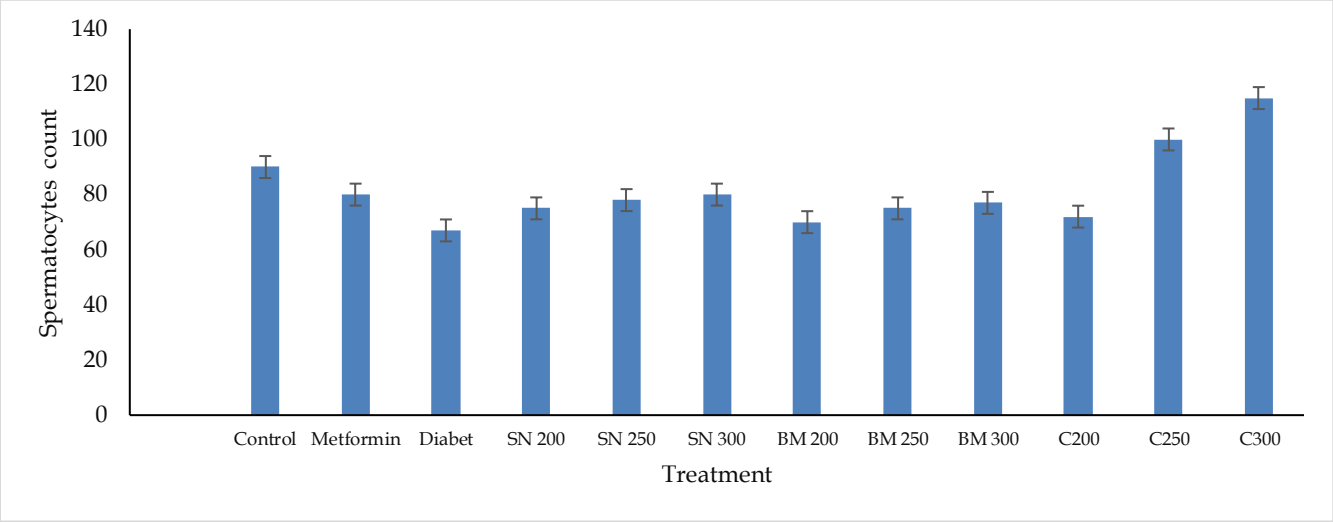

Figure 3. Comparison of the average number of spermatocytes from mice receiving *sambung nyawa* leaves extract, mahogany seeds extract and combined extracts

Spermatid

Figure 4 shows that mice induced with 10% dextrose had a low number of spermatid cells due to high sugar levels without being treated. Mice that received metformin had a higher number of spermatid cells compared to those receiving *sambung nyawa* leaves extract, mahogany seeds extract and combined extracts at a dose of 200 mg/kg BW and mahogany seed extract at a dose of 250 mg/kg BW. The higher the dose of *sambung nyawa* leaves and mahogany seed extracts and the combined extracts, the higher the number of spermatid cells. A significant increase in the number of spermatid cells occurred in the administration of combined extract of *sambung nyawa* leaves and mahogany seeds at a dose of 150:150 mg/kg BW.

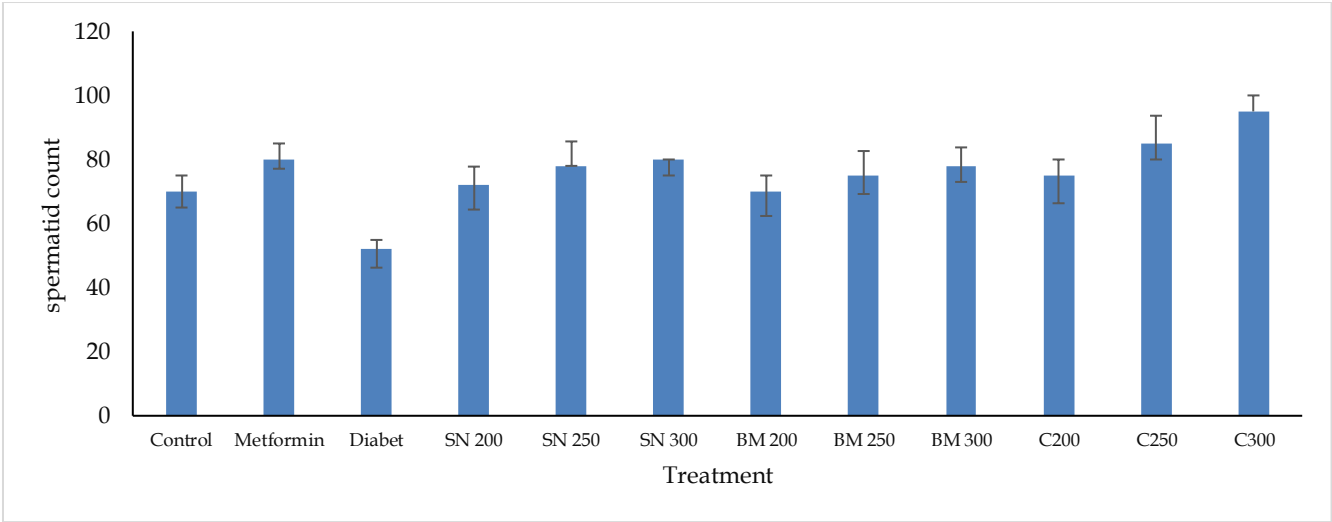

Figure 4. Comparison of the average number of spermatid cells of *sambung nyawa* leaves extract, mahogany seeds extract and combined extracts

Figure 4 shows that mice induced with 10% dextrose had a low number of spermatid cells due to high sugar levels without being treated. Mice that received metformin had a higher number of spermatid cells compared to those receiving *sambung nyawa* leaves extract, mahogany seeds extract and combined extracts at a dose of 200 mg/kg BW and mahogany seed extract at a dose of 250 mg/kg BW. The higher the dose of *sambung nyawa* leaves and mahogany seed extracts and the combined extracts, the higher the number of spermatid cells. A significant increase in the number of spermatid cells occurred in the administration of combined extract of *sambung nyawa* leaves and mahogany seeds at a dose of 150:150 mg/kg BW.

This study found that the number of spermatogonia, spermatocytes and spermatids in diabetic mice induced by 10% dextrose was significantly different from those between control and treatment mice. The study conducted by Adelati et al. (2016) reported that high blood sugar levels in diabetes mellitus can disrupt the stages of spermatogenesis. According to Maresch et al. (2019) reported that hyperglycemia lead to disruption in major glucose metabolism pathways resulting in accumulation of the respective end products within the reproductive tract, which can affect spermatogenesis (Maresch et al., 2019). Huang et al. (2024) state that the complex pathophysiological changes caused by diabetes may induce male infertility in multiple aspects, including hypothalamic pituitary-gonadal axis dysfungtion, spermatogenesis and maturation disorders, testicular interstitial cell damage erectile dysfungtion (Huang et al., 2024). In diabetic rats the mitochondria of spermatogonia show vacuolar changes, the transformation of spermatogonia to primary spermatocytes is decreased, the number of inactive spermatogonia is increased (Zubin et al., 2021).

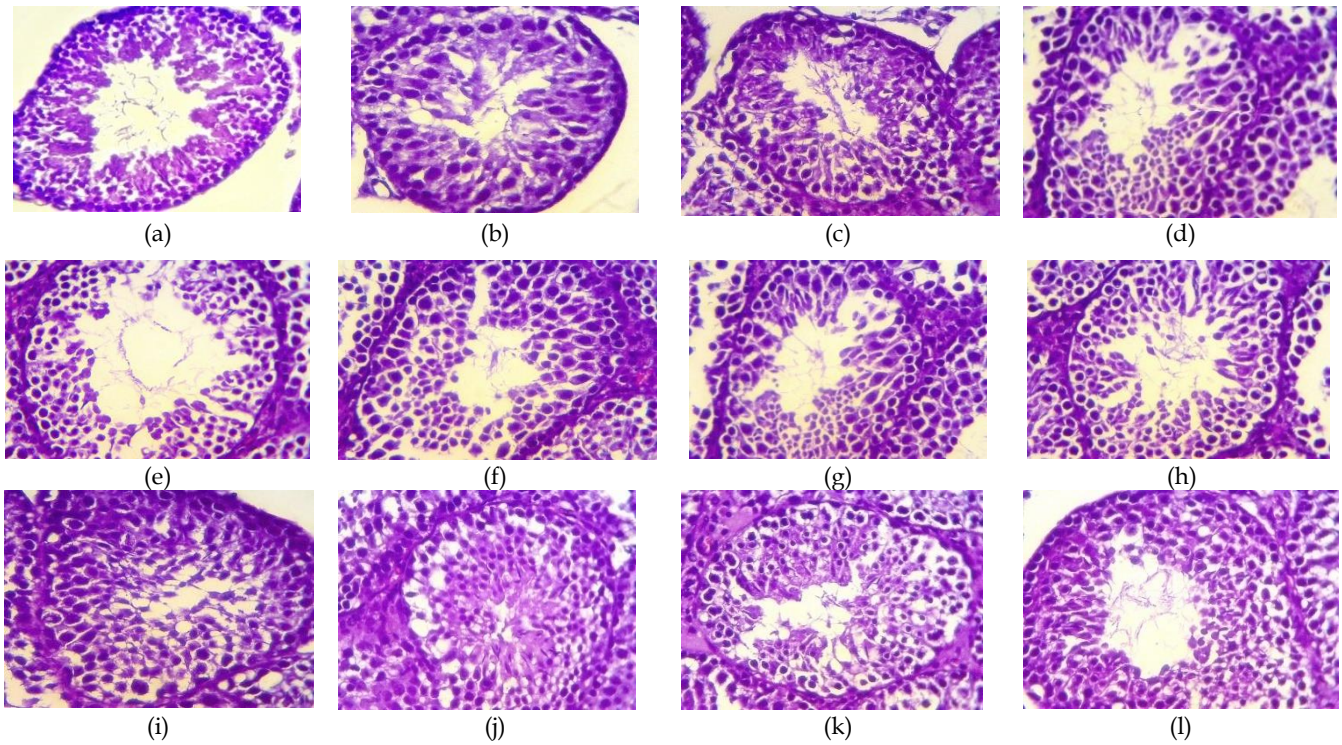

Figure 5. Mice testicular histology: (a) Control; (b) Dexrrose; (c) Metformin; (d) BMA (200); (e) BMB (250); (f) BMC (300); (g) SNA (200); (h) SNB (250); (i) SNC (300); (j) CS (100:100); (k) CB (125:125); and (l) CC (150:150)

Jana et al. (2012) stated that streptozotocin-induced diabetic mice produced significant reductions in testiculo-somatic, epididymal-somatic and seminal vesiculo-somatic indices which might be associated with low serum testosterone levels because testosterone is the main regulator of normal growth of these organs. According to Papadopoulou et al. (2022), in diabetic conditions insulin levels are low. Insulin plays a role in the maintenance of spermatogenesis and testicular endocrine function. Sertoli cells play a role in providing physical and nutritional support for germ cells. Germ cells cannot use glucose as an energy source, but use lactate as a substrate and ATP as an energy source that is used for the spermatogenetic process. Thus, the diabetic condition in mice interferes with the process of spermatogenesis which affects the number of spermatogonia, spermatocytes and spermatids. According to Laleethambika et al. (2019), besides

causing oxidative stress, insulin dysfunction and high availability of glucose, diabetes can also cause severe changes in testicular cells, along with mitochondrial DNA and nuclear fragmentation in spermatogenic cells. Cells will undergo apoptosis and necrosis in circumstances where the testicular environment experiences oxidative stress, resulting in a decrease in the number of spermatogonia, spermatocytes and spermatids. Testicular tissue section of mice shown in Figure 5.

Diabetes lead to abnormal apoptosis of testicular tissue cells and functional damage, and eventually spermatogenic dysfunction (Wang & Wang, 2021). After the administration of *sambung nyawa* leaves extract, mahogany seeds extract and a combination of both extracts for 35 days, the number of spermatogonia, spermatocytes and spermatids increased. This suggests the possibility that the active substances in the extract can repair pancreatic beta cells so that insulin levels increase and oxidative stress decreases, so that ROS can be counteracted by the enzyme catalase, superoxide dismutase or by testicular glutathione. According Guo et al. (2021) reported that the hypoglycemic mechanism of *Gynura procumbens* involves repairing injured islet cells and stimulating the normal secretion of hormones, thereby inhibiting the production of glucose (Guo et al., 2021). Results of studies conducted by Akmar & Noor (2020) that testis histology showed that GPAE (*Gynura procumbens* Aquaous Extract) treated groups produced a significant result whereby the seminiferous tubules were seen packed with sperm and successive stage of spermatogenesis were shown compared to control groups. Hormone analysis suggested that the luteneizing hormone (LH), follicle stimulating hormone (FSH) and testosterone hormone of the treated groups were elevated after seven days of treatment compared to the negative and positive control groups. Tan et al. (2016) state that the Diabetes plant, scientifically named *Gynura procumbens*, is a type of edible herb that exhibits notable anti hyperglycemic properties. This herb is frequently utilized in traditional medicine for the treatment of diabetes due to its hypoglycemic effects. Furthermore, research has revealed that *Gynura procumbens* stimulates the activity of testicular lactate dehydrogenase, an enzyme crucial for spermatogenesis. In diabetic rats, this herb has demonstrated the ability to decrease the percentage of sperm mortality. These findings strongly indicate that *Gynura procumbens* may offer protective benefits for spermatogenic cells in individuals with diabetes (Tan et al., 2016).

According Pusparanee et al. (2016) the extracts of *Gynura procumbens* have the potential to enhance androgen hormone levels, specifically testosterone, in diabetic rats. This improvement may contribute to the regeneration of testicular function and fertility. Furthermore, the administration of *Gynura procumbens* extracts leads to an increase in plasma testosterone levels in diabetic rats. These findings indicate that *Gynura procumbens* can positively influence testosterone levels in the context of diabetes (Pusparanee et al., 2016). The study conducted on male albino rats showed that the methanol extract of *Swietenia mahagoni* seeds had a positive effect on serum testosterone levels. That ethyl acetate extract of *Swietenia mahagoni* had a protective effect on testicular dysfunction induced by diabetes in rats. These findings suggest that *Swietenia mahagoni* may have a potential positive impact on testosterone levels in the context of diabetes, but further research is needed to confirm this effect in humans (Bera & Ghosh, 2018). Santi et al. (2021) reported that 96% ethanol extract of mahogany seeds (Swietenia mahagoni L.) can reduce blood glucose levels in mice induced by alloxan.

Conclusion

This study concluded that administration of the extracts of *sambung nyawa* (*Gynura procumbens*) leaves and mahogany (*Swietenia mahagoni jacq*) seeds and their combinations had the potential to reduce blood glucose levels and increase motility, viability, morphology, concentration, spermatogonia, spermatocytes and spermatid of hyperglycemic mice (*Mus musculus L.*) spermatozoa induced with 10% dextrose.

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Author Contributions
Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation: S, U and D; writing—original draft preparation: S, U and D; writing—review and editing: S; visualization: U,D; supervision: S; project administration: S,U and D; funding acquisition, S,U, and D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

References

Adelati, S., Juniarto, A. Z., & Miranti, I. P. (2016). Histopatologi spermatogenesis testis tikus Wistar diabetes melitus. *DIPONEGORO MEDICAL*

JOURNAL (Jurnal Kedokteran Diponegoro), *5*(4), 1760–1769. https://doi.org/10.14710/dmj.v5i4.15962

Ahmad, A. R., Handayani, V., Syarif, R. A., Najib, A., & Hamidu, L. (2019). *Mahoni (Swietenia mahagoni (L.) Jacq) herbal untuk penyakit diabetes*. CV Nas Media Pustaka: Makassar. Retrieved from https://repository.umi.ac.id/5399/1/BUKU_MAHONI_Fix.pdf

Akmar, K., & Noor, M. M. (2020). The Potential Effect of Gynura Procumbens Aqueous Extract as Anti-Hyperglycaemia , Pro-Fertility and Libido Agent Towards Diabetes-Induced Male Rats. *Proc. Internat.Conf.Sci.Engin*, *3*, 103–108. https://doi.org/10.14421/icse.v3.478

Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules*, *9*(9), 430. https://doi.org/10.3390/biom9090430

Ardiansyah, G., Purwandari, H., & Damayanti, H. P. (2021). Effectiveness of Dry Extract Mahoni Seed Consumption On Blood Sugar Level In Diabetes Mellitus Patients. *International Journal of Nursing and Midwifery Science (IJNMS)*, *5*(1), 70–75. https://doi.org/10.29082/IJNMS/2021/Vol5/Iss1/331

Asadi, N., Bahmani, M., Kheradmand, A., & Rafieian-Kopaei, M. (2017). The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. *Journal of Clinical and Diagnostic Research: JCDR*, *11*(5). https://doi.org/10.7860/JCDR/2017/23927.9886

Asmara, A. P. (2018). A preliminary study of investigating of compound group contained in ethanolic extract of mahagony (Swietenia mahagoni L. Jacq.) seeds related to $α$-glucosidase inhibition. *Jurnal Natural*, *18*(2), 49–56. https://doi.org/10.24815/jn.v18i2.9236

Astuti, A., Antriana, N., & Zelpia. (2017). Biji Mahoni (Swietenia Mahagoni) Menurunkan Glukosa Darah Pada Diabetes Melitus Tipe II. *Jurnal Ipteks Terapan*, *11*(3), 187. https://doi.org/10.22216/jit.2017.v11i3.1964

Auliah, N., & Asri, M. (2019). Uji Aktivitas Sediaan Teh Biji Mahoni (Swietenia Mahagoni) Untuk Menurunkan Kadar Glukkosa dan Aktivitas Peroksidasi Lipid Pada Tikus Diabetes Millitus Tipe 2. *Media Kesehatan Politeknik Kesehatan Makassar*, *14*(2), 191–199. https://doi.org/10.32382/medkes.v14i2.1136

Bera, T. K., & Ghosh, D. (2018). The Corrective Efficacy of the n -Hexane Fraction of the Hydro- Methanol Extract of the Swietenia mahagoni Seeds on Testicular Dysfunctions in Streptozotocin-Induced Diabetic Male Rats. *Jordan Journal of Biological Sciences*, *11*(2), 129–139. Retrieved from https://jjbs.hu.edu.jo/files/v11n2/Paper%20Number%203.pdf

Condorelli, R. A., La Vignera, S., Mongioì, L. M., Alamo, A., & Calogero, A. E. (2018). Diabetes mellitus and infertility: different pathophysiological effects in type 1 and type 2 on sperm function. *Frontiers in Endocrinology*, *9*, 356811. https://doi.org/10.3389/fendo.2018.00268

Ding, G. L., Liu, Y., Liu, M. E., Pan, J. X., Guo, M. X., & Sheng, J. Z. (2015). The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. *AJA, October 2014*, 948–953. https://doi.org/10.4103/1008-682X.150844

Donny Risnanda, H., Cahaya Azzahra, R., & Septia Nurmala, A. Y. (2020). *Potensi Antidiabetes Melitus Senyawa Flavonoid Kulit Jeruk: Sebuah Review Mengenai Mekanisme*. Fakultas Farmasi, Universitas Indonesia. Retrieved from https://bimfi.e-journal.id/bimfi/article/view/48

Dutta, S., Majzoub, A., & Agarwal, A. (2019). Oxidative stress and sperm function : A systematic review on evaluation and management. *Arab Journal of Urology*, *17*(2), 87–97. https://doi.org/10.1080/2090598X.2019.1599624

Fiorenza, M. P., Maslachah, L., Meles, D. K., Widiyatno, T. V, Gandul, A., Ntoruru, J. M., & Luqman, E. M. (2022). Effect of Mahogany (Swietenia mahagoni Jacq .) Extract on the Islet Cells ' Number and Blood Glucose Levels of Alloxan-induced Diabetic Rat. *IJDDT*, *12*(3), 1004–1008. https://doi.org/10.25258/ijddt.12.3.14

Gede, I. P., Purwa, A., Arimbawa, P. E., Putu, N., & Suryaningsih, A. (2022). Characterization and Screening Active Phytochemical Compounds of 70 % Ethanol Extract of Mahogany Seed (Swietenia mahagoni Jacq.) disorders. *DJPS*, *4*(1), 1–8. https://doi.org/10.24252/djps.v4i1.21225

Guardo, F. Di, Vloeberghs, V., Bardhi, E., Blockeel, C., Verheyen, G., Tournaye, H., & Drakopoulos, P. (2020). Low Testosterone and Semen Parameters in Male Partners of Infertile Couples Undergoing IVF with a Total Sperm Count Greater than 5 Million. *Journal of Clinical Medicine*, *9*(3924), 1–9. https://doi.org/10.3390/jcm9123824

Guo, W., Ouyang, H., Liu, M., Wu, J., He, X., Yang, S., & He, M. (2021). Based on Plasma Metabonomics and Network Pharmacology Exploring the Therapeutic Mechanism of Gynura procumbens on Type 2 Diabetes. *Frontiers in Pharmacology 12*, 1–11. https://doi.org/10.3389/fphar.2021.674379

Hakim, P., Sani, H. A., & Noor, M. M. (2008). Effects of

Gynura procumbens extract and glibenclamide on sperm quality and specific activity of testicular lactate dehydrogenase in streptozotocin-induced diabetic rats. *Malaysian Journal of Biochemistry and Molecular Biology*, *16*(2), 10–14.

Harista, R. A., & Lisiswanti, R. (2017). Depresi pada penderita diabetes mellitus tipe 2. *Jurnal Majority*, *4*(9), 73–77.

Huang, R., Chen, J., Guo, B., Jiang, C., & Sun, W. (2024). Diabetes - induced male infertility : potential mechanisms and treatment options. *Molecular Medicine*, *30*(11), 1–15. https://doi.org/10.1186/s10020-023-00771-x

Jana, K., Chatterjee, K., Ali, K. M., De, D., Bera, T. K., & Ghosh, D. (2012). Antihyperglycemic and antioxidative effects of the hydro-methanolic extract of the seeds of Caesalpinia bonduc on streptozotocin-induced diabetes in male albino rats. *Pharmacognosy Research*, *4*(1), 57. https://doi.org/10.4103/0974 8490.91044

Jobaer, A., Ashrafi, S., Ahsan, M., & Hasan, C. M. (2023). Phytochemical and Biological Investigation of an Indigenous Plant of Bangladesh, Gynura procumbens (Lour.) Merr.: Drug Discovery from Nature. *Molecules*, *28*(10), 4186. https://doi.org/10.3390/molecules28104186

Kaewseejan, N., Sutthikhum, V., & Siriamornpun, S. (2015). Potential of Gynura procumbens leaves as source of flavonoid-enriched fractions with enhanced antioxidant capacity. *Journal of Functional Foods*, *12*, 120–128. https://doi.org/10.1016/j.jff.2014.11.001

Kamaruzaman, K. A., Aizat, W. M., & Noor, M. M. (2018). Gynura procumbens improved fertility of diabetic rats: Preliminary study of sperm proteomic. *Evidence-Based Complementary and Alternative Medicine: ECAM*, *2018*(1), 9201539. https://doi.org/0.1155/2018/9201539

Laleethambika, N., Anila, V., Manojkumar, C., Muruganandam, I., Giridharan, B., Ravimanickam, T., & Balachandar, V. (2019). Diabetes and sperm DNA damage: Efficacy of antioxidants. *SN Comprehensive Clinical Medicine*, *1*, 49–59. https://doi.org/10.1007/s42399-018-0012-9

Mannucci, A., Argento, F. R., Fini, E., Coccia, M. E., Taddei, N., Becatti, M., & Fiorillo, C. (2022). The impact of oxidative stress in male infertility. *Frontiers in Molecular Biosciences*, *8*, 799294. https://doi.org/10.3389/fmolb.2021.799294

Maresch, C. C., Stute, D. C., Fleming, T., Lin, J., Hammes, H., & Linn, T. (2019). Hyperglycemia induces spermatogenic disruption via major pathways of diabetes pathogenesis. *Scientific Reports*, *March*, 1–12. https://doi.org/10.1038/s41598-019-49600-4

Naveen, Y. P., Rupini, G. D., Ahmed, F., & Urooj, A. (2014). Pharmacological effects and active phytoconstituents of Swietenia mahagoni: a review. *Journal of Integrative Medicine*, *12*(2), 86–93. https://doi.org/10.1016/S2095-964(14)60018-2

Nurcholis, W., Muthoharoh, R., & Ratu, A. P. (2019). Inhibition of α-glucosidase Activity Seed Extract from Mahogany (Swietenia macrophylla King.). *Current Biochemistry*, *6*(1), 35–44. https://doi.org/10.29244/cb.6.1.7

Papadopoulou, A., Karkalousos, P., & Trapali, M. (2022). Effects of Diabetes Mellitus upon Sperm Quality Insight into Molecular Level. *Journal of Diabetes Mellitus*, *12*(2), 75–86. https://doi.org/10.4236/jdm.2022.122008

Pusparanee, H., Lee, H. W., Halimah, A. S., & Mahanem, M. N. (2016). Effects of Gynura procumbens on Sperm Quality and Testosterone Level in Streptozotocin-induced Type 1 Diabetic Rats. *IJPPR*, *8*(1), 22–30. Retrieved from https://impactfactor.org/PDF/IJPPR/8/IJPPR,Vol8,Issue1,Article5.pdf

Putri, N., & Tjitraresmi, A. (2018). Aktivitas Gynura Procumbens Untuk Terapi Farmakologi: Sebuah Review. *Farmaka Suplemen*, *15*, 213–221. Retrieved from https://jurnal.unpad.ac.id/farmaka/article/view/13303

Santi, W., Eliya, M., & Anisa, M. (2021). Pengaruh Pemberian Ekstrak Etanol 96% Biji Mahoni (Swietenia Mahagoni L.) dalam Menurunkan Kadar Glukosa Darah pada Mencit (Mus Musculus) yang Diinduksi Aloksan. *Jurnal Kedokteran Dan Kesehatan*, *8*(1), 69–74. https://doi.org/10.32539/jkk.v8i1.165

Shiming, Z., Mak, K., Katyayani, M., & Chakravarthi, S. (2021). Biomedicine & Pharmacotherapy Swietenine potentiates the antihyperglycemic and antioxidant activity of Metformin in Streptozotocin induced diabetic rats. *Biomedicine & Pharmacotherapy*, *139*(March), 111576. https://doi.org/10.1016/j.biopha.2021.111576

Sinurat, F. A., & Budi, A. (2023). Uji Efektivitas Antidiabetes Ekstrak Kering Biji Mahoni (Swietenia Mahogani Jacq) Pada Tikus Wistar Jantan Yang Diberikan Diet Tinggi Fruktosa. *Jambura Journal of Health Sciences and Research*, *5*(2), 684–694. https://doi.org/10.35971/jjhsr.v5i2.19130

Smith, L. B., & Walker, W. H. (2014). The regulation of spermatogenesis by androgens. *Seminars in Cell & Developmental Biology*, *30*, 2–13. https://doi.org/10.1016/j.semcdb.2014.02.012

Tan, H. L., Chan, K. G., Pusparajah, P., Lee, L. H., & Goh,

B. H. (2016). Gynura procumbens: An overview of the biological activities. *Frontiers in Pharmacology*, *7*, 182465. https://doi.org/10.3389/fphar.2016.00052

Timotius, K. H., & Rahayu, I. (2020). Overview of herbal therapy with leave of Gynura procumbens (Lour.) Merr. *Journal of Young Pharmacists*, *12*(3), 201. https://doi.org/10.5530/jyp.2020.12.61

Wang, J., & Wang, B. (2021). Effects of Diabetes Mellitus on Sperm Quality in the Db / Db Mouse Model and the Role of the FoxO1 Pathway. *Medical Science Monitor*, *27*, 1–12. https://doi.org/10.12659/MSM.928232

Winata, I. P., & Putri, A. D. (2019). Biji Mahoni sebagai Antioksidan. *Jurnal Penelitian Perawat Profesional*, *1*(1), 89–94. https://doi.org/10.37287/jppp.v1i1.26

Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020). Review Article Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxidative Medicine and Cellular Lonevity*, *2020*, 1–13. https://doi.org/10.1155/2020/8609213

Yudhani, R. D., Aris, D., Nugrahaningsih, A., Sholikhah, E. N., & Mustofa, M. (2021). The molecular mechanisms of hypoglycemic properties and safety profiles of Swietenia macrophylla seeds extract : A review. *Oamjms*, *9*, 370–388. https://doi.org/10.3889/oamjms.2021.6972

Zubin He, Guachao Yin, Qindi Quentin Li, Qiongfang Zeng, J. D. (2021). Diabetes Mellitus Causes Male Reproductive Dysfunction : *In Vivo*, *2511*, 2503–2511. https://doi.org/10.21873/invivo.12531