

The Effect of Tamarind Fruit Nanoparticles (*Tamarindus indica*) on the Sperm Quality of Hyperglycemic Rats

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Abstract

Hyperglycemia is an increase in glucose levels in the blood. This condition can trigger glucose autooxidation which can produce free radicals. Free radicals can cause damage to male reproductive organs and quality. Tamarind fruit has a high flavonoid which has the potential as an antioxidant. This study aims to analyze the effect of tamarind fruit nanoparticles on spermatozoa quality in hyperglycemic rats. This study is experimental with Post-Test Control Group Design. A sample of 15 male Wistar rats were induced by alloxan and then divided into three groups, namely the control group, tamarind fruit nanoparticles with a dose of 30 mg/KgBB (NP1) and 60 mg/KgBB (NP2) for 28 days. Sperm quality was observed with parameters of concentration, motility and viability. The data were analyzed using One Way ANOVA and LSD. The results of the Anova test showed that tamarind nanoparticle treatment could significantly improve sperm quality ($P < 0.05$). The LSD test showed that the concentration, motility and viability of sperm in group (K) were significantly different from groups NP1 and NP2. The highest to lowest average sperm concentration in sequence was in groups NP2 ($49.60 \times 10^6 \pm 6.06 \times 10^6$), NP1 ($41.60 \times 10^6 \pm 7.40 \times 10^6$) and K ($20.80 \times 10^6 \pm 7.56 \times 10^6$). The highest to lowest average percentage of sperm motility in sequence was in groups NP2 (82.20 ± 9.75), NP1 (74.00 ± 4.74), K (47.20 ± 10.66). The highest to lowest average percentage of sperm viability in sequence was in the NP2 group (76.80 ± 4.49), NP1 (72.20 ± 7.12), K (31.60 ± 7.89). The conclusion of this study is that tamarind nanoparticles have an effect on improving sperm quality in hyperglycemic rats.

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INTRODUCTION

People's lifestyle habits that like to consume sweet foods and drinks without paying attention to their composition are one of the factors that must be considered. Sweet foods and drinks generally contain simple carbohydrates or glucose with high glycemic values, so they can increase glucose levels quickly after eating (Tarmizi & Siregar., 2024). This can potentially increase the risk of diabetes mellitus if consumed continuously. Data from the International Diabetes Federation (IDF) shows that Indonesia has a rapidly increasing diabetes rate, with 19.5 million people diagnosed in 2021 and projected to reach 28.6 million in 2045 (IDF, 2021).

Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels or hyperglycemia. Hyperglycemia can cause damage to pancreatic β -cells through an autoimmune process that can interfere with the production of the hormone insulin (Lestari et al., 2021). This causes insulin insufficiency so that glucose levels increase rapidly in the blood. The body will increase glucose metabolism through the glucose autooxidation pathway, glycolysis activity, and sorbitol pathway. These pathways can produce free radicals that can cause damage to various cells due to their highly

reactive nature (Hasan & Yunus., 2023). Diabetes can cause complications that cause neuropathy, nephropathy, angiopathy to the reproductive/gonadal system. This condition raises concerns, especially those related to the health of the reproductive organs in both men and women. Diabetes in men is known to cause infertility by decreasing the quality of sperm produced (Bulqis et al., 2020).

The main factor in infertility and decreased sperm quality in people with hyperglycemia is an increase in free radical compounds, namely reactive oxygen species (ROS) in the body (Elgazar, 2019). The formation of these free radical compounds may arise through various glucose mechanisms, one of which is glucose autooxidation (Adelati et al., 2016). Elevated levels of reactive oxygen species (ROS) can induce cellular damage via lipid peroxidation, as well as oxidative injury to proteins and DNA (Ullah et al., 2016). These deleterious effects may compromise both spermatozoa and mitochondrial membranes, resulting in a reduction in sperm motility and viability (Raad et al., 2024).

Sperm concentration disorders are caused by ROS which can cause disturbances in the sensitivity of the pituitary-gonad glands to gonadotropin hormone (GnRH) stimulation, thereby disrupting the secretion of Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Darbandi et al., 2018). Decreased FSH levels can have an effect on Sertoli cells so that ABP (Androgen Binding Protein) hormone production decreases, which can disrupt the spermatogenesis process. Decreased LH levels in the blood have an effect on Leydig cells in the form of decreased testosterone production. Testosterone deficiency conditions can cause decreased libido, low erection quality, and decreased sperm concentration (Huang et al., 2024).

One of the traditional herbal plants that can provide therapeutic effects on hyperglycemia is tamarind fruit (Asra & Singh., 2024). Tamarind pulp extract at doses of 1, 2, and 3 ml/100 gramsBW of diabetic rats has been shown to increase reproductive hormone secretion and improve spermatozoa quality including concentration, motility and viability in diabetic conditions (Maiti et al., 2017). However, previous studies have highlighted significant challenges with high-dose tamarind extract administration, as therapeutic effects often require large quantities. Moreover, conventional extract formulations are frequently constrained by poor bioavailability and limited cellular uptake (Yusuf et al., 2023). The solution to overcome this problem is to form nanoparticle compounds.

Nanoparticles are more effective than the use of ordinary plant extracts. The use of tamarind fruit nanoparticles at a dose of 50 mg/KgBW in diabetic rats has been shown to reduce blood sugar levels in diabetic rats (Elbaz et al., 2024). Small doses of nanoparticle compounds have the same effect as extracts so they are more effective in treatment. The type of nanoparticle used in biomedicine is silver nanoparticles as a target drug carrier compound (Osman et al., 2024). Nanoparticles have the ability to penetrate the intercellular space so they are more efficient towards target cells (Abdassah, 2017). The size of nanoparticles in drug delivery systems ranges from 50-300 nm (Taurina et al., 2017). This potential underpins the purpose of this research: to analyze the effect of tamarind nanoparticles on the sperm quality of hyperglycemic rats. Scientifically, the research results are expected to provide scientific information regarding the effect of tamarind nanoparticles at specific doses on the sperm quality of hyperglycemic rats, thus providing a basis for further research. The research findings are expected to

develop tamarind content as a biomedical compound capable of naturally controlling diabetes mellitus, thereby increasing public knowledge about tamarind's properties.

METHODS

Extraction and Production of Tamarind Fruit Nanoparticles

Production of tamarind nanoparticles in the Biochemistry Laboratory of the Biology Study Program, FMIPA UNNES. 1 kg of tamarind fruit was separated from its seeds and dried in an oven at 50°C. After drying, the tamarind flesh was ground by blending it into powder and filtered. A total of 20 grams of dry tamarind flesh powder was extracted using 500 ml of 70% ethanol. The solution was homogenized using a magnetic stirrer for 30 minutes. Then macerated for 3 days with stirring every day. The maceration results were filtered using Whatman No.1 paper to produce tamarind fruit extract filtrate.

AgNO₃ solution with a concentration of 1 Mm was made by weighing 0.0850 grams of AgNO₃ powder and then dissolving it in 500 mL of distilled water. The solution was homogenized using a magnetic stirrer for 30 minutes. Synthesis of tamarind nanoparticles was carried out by mixing 40 ml of tamarind fruit extract into 500 ml of silver nitrate solution. The solution was homogenized using a magnetic stirrer for 30 minutes, then put into a microwave oven for 3 minutes. The resulting brown color indicates the formation of AgNP. The tamarind fruit nanoparticles (*Tamarindus indica*) that had been formed were then poured into a tray and dried using an oven at a low temperature for approximately 24 hours. The final result will be the formation of tamarind fruit nanoparticle powder that is ready to be used according to the specified dose.

Treatment of Experimental Animals

A total of 15 male Wistar rats aged 2 months weighing 170-200 grams were acclimatized for 7 days with normal environmental conditions with a temperature of 24°C-26°C and a relative humidity of 70%-80%, a light/dark cycle of about 12 hours, pellet feed and ad-libitum water. Before being treated, the rats were kept for 12 hours. After that, all rats were weighed and induced with alloxan monohydrate at a dose of 125 mg/kg intraperitoneally (Saputri et al., 2021). The rats will receive a 5% sugar solution (dextrose) for the first hour after induction of the alloxan compound. An increase in blood sugar concentration is estimated to occur for 72 hours after induction. Three days after induction, blood sugar levels were measured with the criteria of blood sugar levels > 120 mg/dl to ensure that the rats were in a state of hyperglycemia (Ojiako et al., 2016). The mice were grouped into three treatments, namely the control group (-) or hyperglycemic mice, the tamarind fruit nanoparticle treatment group with a dose of 30mg/Kg/BW (NP1) and 60mg/Kg/BW (NP2). The administration of tamarind fruit nanoparticles was carried out orally for 28 days.

Sperm Stock Solution Collection

At the end of the study, the mice were anesthetized by inhalation anesthesia using chloroform with a concentration of 10% and then surgery was performed on the reproductive organs. The spermatozoa suspension solution was obtained from the cauda epididymis by being rubbed until it

released a white spermatozoa fluid. The spermatozoa fluid was dissolved in 1 ml of 0.9% NaCl solution to obtain a spermatozoa stock solution (Saputri et al., 2021)

Sperm Concentration Calculation

The sperm stock solution was sucked using an erythrocyte pipette to the 0.5 mark. Then with the same pipette, 0.9% NaCl was sucked to the 101 mark. The pipette was shaken slowly so that the mixture of spermatozoa and NaCl became homogeneous. Then, three drops of the mixture were discarded and then dripped into the Neubauer counting chamber and then covered with a cover glass. Observations were carried out microscopically with a magnification of 40X with the provisions of the Neubauer chamber, namely at five points along the diagonal direction (top left, top right, middle, bottom left, bottom right). The concentration of spermatozoa was calculated using the formula counted spermatozoa (S) X dilution factor X multiplication factor (Saputri et al., 2021).

Sperm Motility

The spermatozoa suspension solution was dropped on the object glass as much as 1 drop then covered with a deck glass and observed under a microscope with a magnification of 10x. The calculation of spermatozoa motility was done by calculating the percentage of spermatozoa in 5 fields of view to obtain 100 spermatozoa.

Viability of Sperm

The spermatozoa suspension solution was dropped on the object glass as much as one drop and added one drop of eosin to do a smear. The preparation was covered with a desk glass to be observed using a microscope with a magnification of 40x. Sperm cells that are considered alive have a clear color on the head while dead spermatozoa cells have a reddish color. The calculation was done by observing 100 spermatozoa in five fields of view to obtain the percentage value of live spermatozoa cells.

Data Analysis

Data analysis technique was carried out using Oneway Anova statistical test at a test level of 0.05. Then continued with the Least Significant Differences (LSD) test.

RESULT AND DISCUSSION

The results of the study on spermatozoa quality include concentration, motility in the form of motile (alive) and immotile (dead) spermatozoa and viability treated with tamarind fruit nanoparticles at doses of 30 mg/kgBW and 60 mg/kgBW in hyperglycemic mice are presented in Table 1 as follows:

Table 1. Statistical Calculation Results of Concentration, Motility and Viability of Hyperglycemic Rat Sperm Given Tamarind Nanoparticles

Group	Concentration (Million/ml)	Motility (%)	Viability (%)
K	20.80 ± 7.56 ^a	47.20 ± 10.66 ^a	31.60 ± 7.89 ^a
NP1	41.60 ± 7.40 ^b	74.00 ± 4.74 ^b	72.20 ± 7.12 ^b
NP2	49.60 ± 6.06 ^b	82.20 ± 9.75 ^b	76.80 ± 4.49 ^b

Description: Numbers followed by different letters (a,b) indicate differences ($p < 0.05$). Control treatment (K), Tamarind nanoparticles dose 30 mg/kgBW (NP1), Tamarind nanoparticles dose 60 mg/kgBW (NP2).

Based on the data analysis results using SPSS, it is known that the normality test and homogeneity test on the concentration, motility and viability of spermatozoa show that the data is normally distributed and homogeneous ($p > 0.05$), so that the data meets the requirements of the One Way Anova test. The results of the One Way Anova test on the three parameters showed a significance value of 0.00 ($p < 0.05$) which indicates that the administration of tamarind fruit nanoparticles has a significant effect on improving sperm quality. The analysis was continued using the Post Hoc LSD further test, which showed that the results in the NP1 and NP2 treatment groups had significant differences when compared to the control group (K). However, the two treatment groups did not show any significant differences. This shows that the administration of tamarind fruit nanoparticles has been able to increase the concentration, motility and viability of spermatozoa in hyperglycemic rat.

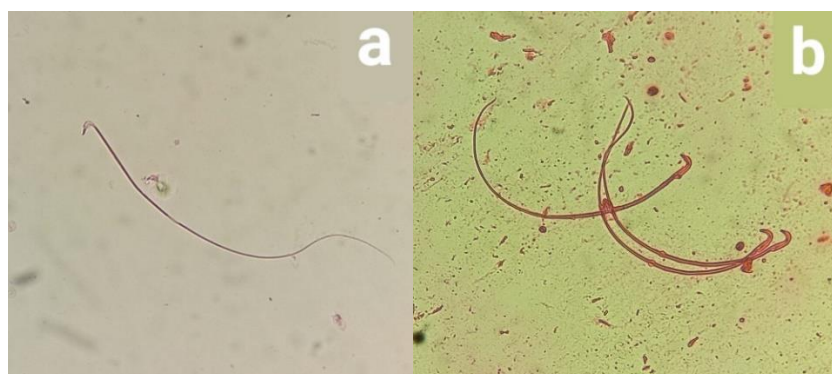


Figure 1. Sperm viability, a) live sperm, b) dead sperm (400x magnification with eosin staining)

Diabetes mellitus is a chronic metabolic disorder characterized by increased blood glucose levels or hyperglycemia. In diabetes, pancreatic β -cells can be damaged through several mechanisms. In type 1 diabetes, damage to pancreatic β -cells is caused by an autoimmune process so that insulin hormone secretion decreases (Lestari et al., 2021). In type 2 diabetes, pancreatic β -cells experience stress as a result of insulin resistance which causes increased work of pancreatic β -cells to secrete insulin or hyperinsulinemia (Dludla et al., 2023). This damage causes glucose in the blood to be unable to enter the cells so that there will be an increase in blood flow. This condition can cause glucose in the blood to be oxidized spontaneously (autooxidation) which can cause the formation of free radicals.

The mechanism of decreased sperm quality in hyperglycemic conditions is due to increased formation of free radicals through the glucose autooxidation pathway. In the glucose autooxidation pathway, it begins with the oxidation of the α -hydroxyaldehyde part of glucose which will form enediol (Ramos-Riera et al., 2023). The presence of transition metals such as Fe^{3+} can act as catalysts that can accelerate the oxidation reaction. This reaction will produce Fe^{2+} which when reacted with oxygen molecules (O_2) can produce superoxide anions ($\text{O}_2^{\bullet-}$) and Fe^{3+} ions again. Superoxide anions ($\text{O}_2^{\bullet-}$) as free radicals in the body will undergo dismutation by SOD which can produce hydrogen peroxide (H_2O_2). If hydrogen peroxide (H_2O_2) reacts with Fe^{2+} or Cu^{2+} metal, hydroxyl radicals ($\bullet\text{OH}$) will be formed through the Fenton reaction and if it reacts with other radical compounds such as superoxide anions ($\text{O}_2^{\bullet-}$) it can form hydroxyl radicals ($\bullet\text{OH}$) through the Haber-Weiss reaction (González et al., 2023). Hydroxyl radicals ($\bullet\text{OH}$) are highly reactive free radicals that can cause lipid oxidation.

Free radicals (ROS) are known to cause impaired pituitary-pituitary-gonadal sensitivity to gonadotropin hormone (GnRH) stimulation, which results in impaired secretion of LH and FSH hormones (Maiti et al., 2017). Free radicals cause lipid peroxidation by damaging membranes and disrupting hormone receptor function in gonadotroph cells in the pituitary gonads. Hormone deficiency during spermatogenesis can disrupt spermatozoa formation. Free radicals can disrupt spermatogenesis in the testes by damaging the blood-testis barrier (BTB) (Papadoupulou et al., 2022). ROS can damage tight junctions between Sertoli cells through lipid peroxidation. This damage mechanism also occurs in Leydig cells, disrupting cholesterol transfer in the mitochondria, which can inhibit testosterone formation (Walke et al., 2023).

Sperm cells are highly susceptible to ROS damage due to the unique characteristics of their membranes. The membrane's main component is composed of polyunsaturated fatty acids (PUFAs), which have a high affinity for ROS, making them highly sensitive and readily bind to ROS. Sperm also have a limited antioxidant enzyme system (Walke et al., 2023). This leads to lipid peroxidation, which can damage the plasma membrane and mitochondrial membranes of spermatozoa (Wijayanti & Lestari, 2018). Damage to the mitochondrial membrane prevents spermatozoa from producing ATP as energy, thus disrupting sperm motility. In observing spermatozoa viability, spermatozoa cells that are alive in the head will not absorb dyes and will be clear, while spermatozoa cells that are dead in the head will absorb dyes and be red. This is to damage the sperm membrane due to lipid peroxidation which can reduce membrane integrity so that dyes can easily enter the sperm cell head membrane (Arundani et al., 2021).

Tamarind nanoparticles can increase the concentration of sperm cells. Tamarind fruit (*Tamarindus indica*) contains antioxidant compounds that can neutralize free radicals and can protect gonadotroph cells in the pituitary gonad and Sertoli cells and Leydig cells in the testes from damage caused by ROS. Compounds that can act as antioxidants are flavonoids. Possible types of flavonoids that have a role as antioxidants are flavonoids of the quercetin and catechin types (Asra & Singh, 2024). Quercetin and catechin are known as key antioxidants that can neutralize free radicals by preventing the transfer of hydrogen (H) to them. These compounds contain a carbon (OH) group bound to an aromatic carbon ring that can donate a hydrogen (H) atom, thereby neutralizing free radicals. These compounds stabilize free radicals (ROS) (Zhang et al., 2023). Quercetin is known to neutralize free radicals (ROS) in the testes, reduce lipid peroxidation, and increase sperm count (Mustafa, 2023).

Luteolin in tamarind fruit has the potential to increase Leydig cell steroidogenesis. Luteolin can increase the StAR (Steroidogenic Acute Regulatory Protein) gene, which functions in cholesterol transport from the outer to the inner mitochondrial membrane. Increasing the StAR gene can enable the P450_{scc} enzyme to catalyze the breakdown of cholesterol side chains and produce pregnenolone, the main precursor of steroid hormones, including testosterone (Martin & Touaibia, 2020). Luteolin are also able to improve Leydig cell function through the cAMP-dependent protein kinase-A (cAMP/PKA) signaling pathway by inhibiting Cyclooxygenase-2 (COX-2) signal transduction (Courtoure et al., 2020). COX-2 is an enzyme that is usually active during inflammation which can interfere with normal cell function.

Tamarind nanoparticles are also able to increase spermatozoa motility and viability. Antioxidant compounds contained in tamarind fruit that can neutralize free radicals are flavonoids of the quercetin and catechin types known to have the ability to neutralize free radical compounds by stopping the transfer of hydrogen (H) in radical compounds (Asra & Singh, 2024). Quercetin can neutralize ROS, reduce lipid peroxidation, and increase sperm cell motility and viability (Mustafa, 2023).

Quercetin is known to regenerate pancreatic β -cell damage caused by hyperglycemia. Free radicals cause apoptosis in pancreatic β -cells through lipid peroxidation (Ansari et al., 2023). Glutathione peroxidase (GPX) is an endogenous antioxidant that can protect cells from lipid peroxidation and suppress apoptosis in pancreatic β -cells. Quercetin can increase GPX activity in the pancreas, reduce oxidative stress, and increase insulin production and secretion by pancreatic β -cells (Li et al., 2020). Quercetin can also reduce TNF- α levels by inhibiting NF- κ B. NF- κ B plays a role in controlling the expression of genes encoding pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and other proteins. Inhibiting NF- κ B activation will attenuate the inflammatory response, thereby inhibiting pancreatic β -cell damage (Rifaa et al., 2015). The quercetin compound is able to inhibit the release of pro-inflammatory mediators such as IL-1, IL-6, IL-8, IL-4, and TNF- α so that it can prevent damage to pancreatic β cells (Ansari et al., 2023).

Tamarind fruit silver nanoparticles are an innovation in effective drug delivery because of their small size so they are easily absorbed by the body (Yusuf et al., 2023). The size of nanoparticles as a drug delivery medium has a size ranging from 50-350 which can increase effectiveness with its ability to penetrate intercellular spaces so that it is more efficient towards target cells (Taurina et al., 2017). The use of compounds contained in tamarind fruit as reducing agents such as polyphenols, flavonoids, and flavones can improve the properties of silver nanoparticles and can reduce toxicity caused by the use of metal compounds (Ali et al., 2023). The antioxidant ability of tamarind silver nanoparticles is expected to delay, slow down and prevent the oxidation process by binding free radicals so that it can reduce ROS levels and improve sperm quality (Patabang et al., 2019).

CONCLUSION

Tamarind fruit nanoparticle treatment (*Tamarindus indica*) has an effect on increasing the quality of spermatozoa in hyperglycemic rats. Tamarind nanoparticles were able to increase spermatozoa cell concentration and the percentage of motile and live sperm. The highest average sperm concentration sequence was in groups NP2 ($49.60 \times 10^6 \pm 6.06 \times 10^6$), sperm motility (82.20 ± 9.75) and sperm viability (76.80 ± 4.49). However, sperm quality cannot be determined based on concentration, motility, and viability alone. Therefore, it is necessary to measure other indicators such as sperm morphology, testicular histopathology, and reproductive hormone levels such as testosterone. Having demonstrated positive results in rats, this research needs to be further developed in the pre-clinical stage to assess its consistency and potential in the biomedical field.

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