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Potential of butterfly pea (*Clitoria ternatea*) nanoherbal on spermatozoa quality in hyperglycemic rats

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Abstract

Hyperglycemia is a condition characterized by elevated blood glucose levels that induce free radical formation, leading to reduced spermatozoa quality. Butterfly pea (*Clitoria ternatea*) contains antioxidant compounds and is formulated as a nanoherbal to enhance bioavailability. This study aimed to evaluate the potential of butterfly pea nanoherbal in improving spermatozoa quality in hyperglycemic rats. This experimental study employed a post-test only control group design using 25 male Wistar rats divided into a healthy control group and hyperglycemic groups induced by alloxan (125 mg/kg BW). The hyperglycemic groups consisted of a metformin control (400 mg/kg BW) and butterfly pea nanoherbal treatment groups at doses of 12.5, 25, and 50 mg/kg BW administered for 28 days. Data were analyzed using One-Way ANOVA followed by the Least Significant Difference (LSD) post hoc test. The results showed that spermatozoa concentration in the healthy control group differed significantly from the positive control, P1, and P2 groups ($p<0.05$), but not from the P3 group. Spermatozoa motility and viability also showed significant differences among groups ($p<0.05$). It can be concluded that butterfly pea flower nanoherbal significantly improves spermatozoa quality in hyperglycemic rats.

Introduction

Uncontrolled modern dietary patterns characterized by high caloric intake, excessive sugar and saturated fat consumption, and low intake of fiber, vitamins, and minerals can increase the risk of degenerative diseases (Musta'in et al., 2023). Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, defined as elevated blood glucose levels resulting from impaired insulin secretion or insulin action (Budianto et al., 2022). Chronic hyperglycemia may cause damage to pancreatic β cells, which are responsible for insulin production, and contribute to the development of insulin resistance. Under these conditions, the body produces excessive free radicals, particularly reactive oxygen species (ROS) (Papadopoulou et al., 2022).

Excessive free radical production adversely affects the reproductive system, particularly in males, by disrupting the process of spermatogenesis. Decreased spermatozoa quality can be evaluated through parameters such as spermatozoa concentration, motility, and viability. Accumulated free radicals may impair spermatozoa quality by affecting the sensitivity of hypothalamic cells, leading to reduced secretion of gonadotropin-releasing hormone (GnRH), which subsequently inhibits the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland (Darbandi et al., 2018). Reduced FSH levels interfere with spermatogenesis in the testes, while decreased LH levels affect testosterone production, which plays an essential role in the stages of spermatogenesis (Suarni et al., 2021).

Spermatogenesis is a complex process involving the formation of male gametes within the seminiferous tubules (Talin et al., 2024). Excessive ROS levels can damage the axonemal protein structure, a critical component of the spermatozoa tail, resulting in decreased sperm motility (Pereira et al., 2017). In addition, ROS can damage the spermatozoa cell membrane, affecting membrane integrity, fluidity, and function, which ultimately leads to apoptosis and a reduction in spermatozoa number (Hoesada et al., 2016). Previous studies by Suarni et al, (2021) demonstrated that oxidative stress under hyperglycemic conditions can reduce sperm quality through decreased hormone production, sperm membrane damage, and DNA fragmentation. Furthermore, Papadopoulou et al, (2022) reported that ROS reduce sperm quality through sperm DNA fragmentation and lipid peroxidation.

Diabetes mellitus is a chronic disease that cannot be cured but can be managed through lifestyle modifications, such as regular physical activity, dietary improvement, and the use of chemical or herbal medications. Pharmacological treatments, including chemical drugs such as metformin, are widely used however, they are associated with several adverse effects, including gastrointestinal disturbances, hypoglycemia, tremors, and vitamin B12 malabsorption (Putra et al., 2017). Therefore, the use of herbal plants in traditional medicine has increased due to their relatively minimal side effects, one of which is butterfly pea (*Clitoria ternatea*). Phytochemical screening studies conducted by Cahyaningsih et al, (2019) revealed that butterfly pea flowers contain secondary metabolites such as flavonoids, saponins, terpenoids, and tannins. These compounds exhibit high antioxidant activity (Dewanti et al., 2023) reported that butterfly pea flower nanoparticles possess strong antioxidant activity, with a value of 6.97 ± 0.082. Although numerous studies have reported that hyperglycemic conditions can reduce spermatozoa quality through oxidative stress mechanisms, and evidence has shown that butterfly pea flower possesses potent antioxidant properties, studies investigating the application of butterfly pea flower in nanoherbal formulations to improve spermatozoa quality in hyperglycemic rats remain limited. Previous research has predominantly focused on extracts or conventional formulations, which have several limitations, including low bioavailability, poor stability, and reduced gastrointestinal absorption (Dewanti et al., 2023).

Therefore, it is necessary to develop formulations in the form of nanoparticles or nanoherbals with a particle size range of 50–500 nm (Samudra et al., 2021). Nanoherbal formulations offer several advantages over conventional extracts, including improved stability, higher bioavailability, and enhanced delivery of active compounds, thereby enabling more effective absorption to target organs (Dwitarani et al., 2021). Based on these considerations, this study aimed to determine whether the administration of butterfly pea flower nanoherbal (*Clitoria ternatea*) has the potential to improve spermatozoa quality, including concentration, motility, and viability, in hyperglycemic rats.

Materials and methods

1. Research subject

The research subjects were 25 male Wistar rats aged 8 weeks with body weights of 170–200 g, having smooth fur, no baldness, no anatomical abnormalities, and normal activity.

2. Materials

The instruments used in this study included an analytical balance, blender, glassware, magnetic stirrer, 40-mesh sieve, maceration vessel, mixer, rotary evaporator, hot plate, sonicator, Particle Size Analyzer (PSA), syringe, glucometer and test strips, oral gavage, surgical instruments, petri dishes, pipettes, hemocytometer, binocular microscope, hand tally counter, and camera. The materials used consisted of butterfly pea (*Clitoria ternatea*) flowers obtained from Bandungan Market, Semarang Regency, ethanol p.a, ethanol 96%, Whatman No. 1 filter paper; sodium alginate 0.1%, $CaCl_2$ 0.02%, distilled water; male Wistar rats, alloxan monohydrate, metformin, ketamin, 0.9% NaCl solution, eosin, microscope slides, and cover glasses.

3. Method and research design

This study employed an experimental method using a completely randomized design (CRD) with a post-test only control group design. The observed variables were spermatozoa quality parameters, including concentration, motility, and viability. The experimental animals were divided into five groups: a healthy control group, a positive control group receiving metformin at a dose of 400 mg/kg BW, and three

treatment groups administered butterfly pea nanoherbal at different doses, namely P1 (12.5 mg/kg BW), P2 (25 mg/kg BW), and P3 (50 mg/kg BW).

4. Procedures

1. Preparation of Butterfly Pea Nanoherbal (*Clitoria ternatea*)

Dried butterfly pea flowers were ground using a blender and sieved through a 40-mesh sieve. Extraction was carried out using 200 g of butterfly pea simplicia powder placed in a maceration vessel and mixed with 600 mL of 96% ethanol (Fadel et al., 2024). The mixture was left for 24 hours with stirring performed three times, then filtered using Whatman No. 1 filter paper. The residue was remacerated once. All obtained filtrates were evaporated using a rotary evaporator to obtain a thick extract.

A total of 500 mg of the thick extract was dissolved in 2.5 mL of ethanol p.a, and distilled water was added to a final volume of 50 mL. The butterfly pea nanoherbal was prepared using a formulation ratio of extract : sodium alginate : $CaCl_2$ of 1 : 1 : 5 (Rohmahdana et al., 2024). One milliliter of butterfly pea extract solution and 1 mL of 0.1% sodium alginate were placed into a beaker and stirred for 30 minutes at 1500 rpm. Subsequently, 5 mL of 0.02% $CaCl_2$ was added gradually. After the mixture became homogeneous, sonication was performed for 60 minutes. The nanoherbal suspension obtained after sonication had a concentration of 10 mg/mL and was stored at 4 °C. Particle size analysis was then conducted using a Particle Size Analyzer (Horiba SZ-100). The PSA results showed that the butterfly pea nanoherbal particle size was 350.5 nm.

2. Experimental Animal Treatment

Adult male Wistar rats weighing 170–200 g was acclimatized for 7 days under controlled conditions, including a temperature of 24–26 °C, relative humidity of 70%, and a 12 h light: 12 h dark cycle. After acclimatization, the rats were grouped according to their respective treatments, including a healthy control group, a positive control group, and nanoherbal treatment groups. Alloxan monohydrate at a dose of 125 mg/kg BW was administered intraperitoneally to the positive control group and the nanoherbal treatment groups. Hyperglycemic status was confirmed three days after induction, defined by blood glucose levels >150 mg/dL. Subsequently, treatments were administered for 28 days according to group allocation: healthy control, positive control (metformin 400 mg/kg BW), and butterfly pea nanoherbal treatment groups at doses of 12.5 mg/kg BW (P1), 25 mg/kg BW (P2), and 50 mg/kg BW (P3).

3. Data Collection

Experimental animals were anesthetized with ketamine and surgically dissected to collect the cauda epididymis. The cauda epididymis was placed in a petri dish containing 1 mL of 0.9% NaCl solution and gently minced. The spermatozoa suspension was homogenized in 1 mL of 0.9% NaCl (Syahputra et al., 2019). The resulting sperm suspension was used for the evaluation of sperm quality parameters, including concentration, motility, and viability.

4. Sample Preparation

Spermatozoa Concentration

The sperm suspension was aspirated into a hemocytometer pipette up to the 0.5 mark, followed by aspiration of physiological NaCl solution up to the 101 marks. The pipette tip was closed and the suspension was homogenized by gently shaking in a figure-eight motion. An Improved Neubauer counting chamber covered with a cover glass was prepared and examined under a microscope until the grid lines were visible. One drop of sperm suspension was placed at the edge of the cover glass and allowed to spread evenly. Spermatozoa concentration was counted in five squares (four corner squares and one central square) under 40× magnification. Spermatozoa concentration was calculated using the following formula; Spermatozoa concentration (million/mL suspension) = number of spermatozoa × 10,000 × dilution factor (200) (Saputri et al., 2021).

Spermatozoa Motility

One drop of sperm suspension was placed on a microscope slide and covered with a cover glass. Observations were performed under 10× magnification, and spermatozoa were classified as motile or

immotile. Motility was calculated based on 100 spermatozoa and expressed as a percentage (%) (Ihsani et al., 2019).

Spermatozoa Viability

Viability assessment was performed by placing one drop of sperm suspension on a microscope slide and adding one drop of eosin stain. A smear preparation was made and air-dried. Observations were conducted under 40× magnification. Live spermatozoa were indicated by heads that did not absorb the red stain (transparent), whereas dead spermatozoa showed red-stained heads (Khotijah & Rusmiati, 2024). A total of 100 spermatozoa were counted and viability was expressed as a percentage (%).

Data Analysis

The collected data were analyzed using one-way ANOVA followed by the Least Significant Difference (LSD) post hoc test to determine differences among groups. Statistical analysis was performed using SPSS software.

Ethical Approval

All experimental procedures involving animals were approved by the Ethics Committee of the Faculty of Medicine, Universitas Diponegoro, with approval number 132/EC/KEPK/FK-UNDIP/VI/2025.

Result and discussion

The results of spermatozoa quality assessment, including concentration, motility, and viability, following treatment with nanoherbal *Clitoria ternatea* extract at doses of 12.5 mg/kg BW, 25 mg/kg BW, and 50 mg/kg BW in hyperglycemic rats are presented in Table 1.

Table 1. Mean ± SD (SE) of Spermatozoa Concentration, Motility, and Viability in Control and Treatment Groups

Group	Mean ± SD (SE)		
	Concentration ($\times 10^6$/mL)	Motility (%)	Viability (%)
K	44.4 ± 7.92^{c} (3.54)	83.20 ± 5.63^{d} (2.52)	85.60 ± 3.04^{d}(1.36)
K+	30.0 ± 3.16^{b} (1.41)	58.80 ± 6.87^{b} (3.07)	56.60 ± 2.40^{b} (1.07)
P1	16.8 ± 4.60^{a} (2.06)	32.80 ± 3.83^{a} (1.71)	30.60 ± 6.91^{a} (3.09)
P2	28.4 ± 2.61^{b} (1.17)	57.00 ± 5.52^{b} (2.47)	53.40 ± 6.65^{b} (2.97)
P3	39.2 ± 9.96^{c} (4.45)	70.20 ± 3.11^{c} (1.39)	72.00 ± 3.16^{c} (1.41)

Note: Values followed by different superscript letters (a, b, c, d) indicate a significant difference between groups ($p < 0.05$)

The analysis showed that variations in nanoherbal(*Clitoria ternatea*) doses exerted different effects on spermatozoa concentration, motility, and viability. Spermatozoa concentration in the healthy control group (K) and P3 showed no significant difference, while the positive control group (K+) and P2 also showed no significant difference ($p > 0.05$). The highest to lowest spermatozoa concentrations were observed sequentially in groups K, P3, K+, P2, and P1. The analysis of motility and viability demonstrated a similar pattern, in which groups K+ and P2 showed no significant difference ($p > 0.05$). The highest to lowest motility and viability values were observed in groups K, P3, K+, P2, and P1, respectively.

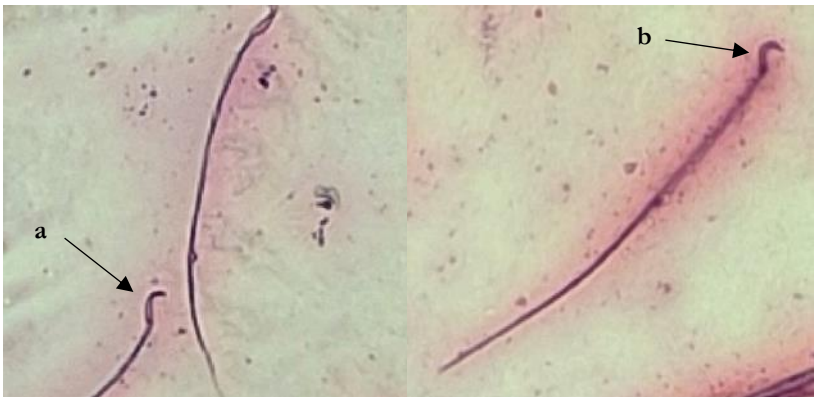

Figure 1. Observation of spermatozoa viability. (a) Live spermatozoa showing a transparent (unstained) sperm head. (b) Dead spermatozoa showing a red-stained sperm head.

Microscopic observations presented in Figure 1(a) show spermatozoa heads that did not absorb the red stain and appeared transparent, indicating viable spermatozoa. In contrast, Figure 1(b) shows spermatozoa heads that absorbed the red stain, indicating non-viable spermatozoa.

The results in alloxan-induced groups treated with nanoherbal butterfly pea at doses of 12.5 mg/kg BW, 25 mg/kg BW, 50 mg/kg BW, and metformin at 400 mg/kg BW demonstrated spermatozoa quality (concentration, motility, and viability) that remained lower compared to the healthy control group. The reduction in spermatozoa concentration is associated with hyperglycemic conditions that increase reactive oxygen species (ROS) production. ROS accumulation contributes to impaired spermatozoa quality and disrupts the sensitivity of the hypothalamic–pituitary–gonadal axis (HPGA) to gonadotropin-releasing hormone (GnRH). Impaired GnRH secretion leads to decreased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, thereby affecting spermatogenesis. Reduced LH and FSH levels directly impact spermatozoa concentration. FSH stimulates Sertoli cells to produce androgen-binding protein (ABP), which transports testosterone from Leydig cells to the seminiferous tubules and plays a critical role in spermatogenesis (Sanaky et al., 2024). LH stimulates Leydig cells to produce testosterone, an essential hormone for spermatogenic cell development and spermatozoa formation (Sukarjati et al., 2024). Therefore, reductions in LH, FSH, and testosterone directly contribute to decreased spermatozoa concentration.

In addition, ROS accumulation disrupts spermatogenesis through structural damage to testicular tissue. Spermatogenesis is a complex process involving the differentiation of spermatogonia into spermatozoa through mitotic and meiotic stages within Sertoli cells. Sertoli cells are interconnected by tight junctions that form the blood–testis barrier (BTB), which maintains the microenvironment of germ cells by protecting them from immune exposure and toxic molecules, thereby ensuring normal spermatogenesis (Washburn et al., 2022). Elevated ROS levels can damage BTB tight junctions, causing barrier leakage and disruption of the germ cell microenvironment. ROS also damages the Sertoli cell cytoskeleton, leading to structural fragility, nuclear dislocation, DNA fragmentation, and apoptosis (Wahyuningsih et al., 2016). Damage to Sertoli cells impairs their nutritional and structural support for germ cells, thereby disrupting spermatogenesis. Similar mechanisms may occur in Leydig cells, where oxidative stress inhibits cholesterol transport into mitochondria, resulting in impaired testosterone synthesis (Wahyuningsih et al., 2016). Reduced testosterone levels further deteriorate spermatozoa quality and maturation.

Excessive ROS production without sufficient antioxidant defense reduces testosterone secretion, leading to decreased secretion of factors supporting spermatozoa maturation. Consequently, spermatozoa in the epididymis are unable to initiate progressive motility or maintain viability (Suarni et al., 2021). The motility results showed that all alloxan-induced groups receiving nanoherbal or metformin treatment differed significantly from the healthy control group. Physiologically, progressive motility is initiated after newly formed spermatozoa are released into the seminiferous tubule lumen in an immature state and subsequently enter the epididymis to undergo maturation. During epididymal transit, spermatozoa undergo biochemical and structural modifications necessary to acquire progressive motility before fertilization capability. Mature spermatozoa are highly susceptible to ROS-induced damage because their membranes are rich in polyunsaturated fatty acids (PUFA), which are easily oxidized, leading to lipid peroxidation. This

susceptibility is exacerbated by the relatively low endogenous antioxidant capacity of spermatozoa, which is insufficient to neutralize ROS effectively (Walke et al., 2023).

Excessive accumulation of reactive oxygen species (ROS) in spermatozoa mitochondria leads to a reduction in sperm motility. Elevated ROS levels induce oxidative stress that damages the inner mitochondrial membrane (IMM) (Costa et al., 2023). Damage to the IMM results in a decrease in mitochondrial membrane potential (MMP), thereby disrupting oxidative phosphorylation and reducing adenosine triphosphate (ATP) production. ATP is essential for flagellar movement through coordinated contraction and relaxation patterns, enabling spermatozoa motility. Papadopoulou et al, (2022) reported that diabetes mellitus can damage more than 45 mitochondria within a single spermatozoon. Such mitochondrial damage interferes with oxidative phosphorylation, leading to decreased ATP production (Hoesada et al., 2016). In addition, ROS can trigger lipid peroxidation of the mitochondrial membrane, which is rich in polyunsaturated fatty acids (PUFAs), resulting in the formation of reactive aldehydes. These aldehydes inhibit electron transport chain proteins and promote repetitive ROS generation, thereby exacerbating mitochondrial dysfunction.

Furthermore, excessive ROS induces mitochondrial DNA (mtDNA) damage, which further impairs electron transport efficiency and reduces energy production. This condition may activate intrinsic or truncated apoptotic pathways through the opening of the mitochondrial permeability transition pore, reduction of MMP, and activation of caspases (Costa et al., 2023). Overall, ROS accumulation in mitochondria causes mitochondrial dysfunction, energy deficiency, and cellular apoptosis, all of which contribute to decreased spermatozoa motility. In addition to mitochondrial dysfunction, progressive motility and spermatozoa capacitation are influenced by substances secreted by the epididymis, including Ca^{2+}, Na^{+}, K^{+}, and Cl^{-} ions, proteins, sialic acid, glycogen, lactic acid, phospholipids, as well as enzymes such as lactate dehydrogenase (LDH) and acid and alkaline phosphatases. Testosterone also plays a critical role in epididymal maturation (Adriani & Nita, 2015). Hormonal imbalance due to hyperglycemia, particularly decreased testosterone levels, further exacerbates impaired spermatozoa motility.

A decrease in spermatozoa viability in groups P1, P2, and P3 was observed based on differences in sperm head staining. In Figure 1(a), spermatozoa heads that did not absorb the dye appeared transparent, indicating viable spermatozoa. In contrast, Figure 1(b) shows non-viable spermatozoa whose heads absorbed the red dye. The increased number of red-stained sperm heads indicates plasma membrane damage caused by oxidative stress and lipid peroxidation. Lipid peroxidation of the sperm membrane is initiated by hydroxyl radicals that abstract hydrogen atoms from PUFA components, producing unstable lipid radicals that react with oxygen to form lipid peroxyl radicals. These radicals propagate chain reactions that further damage membrane structure, disrupt membrane integrity and fluidity, and allow dye penetration into the sperm head (Arundani et al., 2021). In addition to membrane damage, ROS can directly attack sperm DNA, causing denaturation and fragmentation that ultimately leads to cell death. Decreased testosterone levels also contribute to reduced sperm viability, as testosterone plays a key role in maintaining sperm viability during epididymal maturation (Adriani & Nita, 2015).

Administration of nanoherbal *Clitoria ternatea* at different doses influenced spermatozoa quality in hyperglycemic rats. The analysis showed that the mean values in group P3 (50 mg/kg BW) approached those of the healthy control group, while P2 (25 mg/kg BW) and the positive control group showed no significant difference ($p > 0.05$). These findings indicate that nanoherbal *Clitoria ternatea* treatment can improve spermatozoa concentration, motility, and viability in hyperglycemic rats. The bioactive compounds in butterfly pea are biomolecules with high biological activity that serve as natural antioxidants.

Flavonoids in *Clitoria ternatea* influence spermatozoa concentration by acting as natural antioxidants that donate hydrogen atoms to neutralize free radicals, thereby protecting gonadotropic cells, Leydig cells, and Sertoli cells from oxidative damage. In addition, flavonoids and saponins contribute to tissue regeneration, protection of pancreatic β cells, and increased insulin sensitivity, which subsequently supports Sertoli cell metabolic function during spermatogenesis. Flavonoids also improve spermatozoa motility by protecting sperm mitochondria from ROS induced damage, which is essential for maintaining ATP production in the midpiece as the primary energy source for flagellar movement. Increased ATP availability enhances progressive motility. Moreover, reduced ROS levels prevent lipid peroxidation of the flagellar membrane, preserve axonemal structural stability, and support epididymal maturation, which is highly dependent on hormonal stability and an optimal physiological environment (Mishra et al., 2024).

At the molecular level, the improvement in spermatozoa quality observed in the butterfly pea flower nanoherbal treated groups is associated with the ability of flavonoids and phenolic compounds to suppress intracellular ROS production, thereby inhibiting the activation of mitochondria mediated intrinsic apoptotic pathways. Reduced oxidative stress prevents cytochrome c release and subsequent activation of caspase 9 and caspase 3, which play critical roles in germ cell death. Preservation of mitochondrial integrity and sperm DNA integrity allows spermatogenesis and sperm maturation processes to proceed more optimally. These combined effects contribute to improved sperm motility in the treatment groups, particularly in P3, which exhibited mean values closest to the healthy control group.

Saponins in *Clitoria ternatea* also play a crucial role in reducing oxidative stress in testicular tissue by enhancing antioxidant enzyme activity, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), and by reducing malondialdehyde (MDA) levels as a marker of lipid peroxidation (Pashapour et al., 2023). Increased antioxidant capacity prevents damage to cell membranes, DNA, and proteins in germ cells and Leydig cells. Furthermore, saponins upregulate the expression of steroidogenic genes, including steroidogenic acute regulatory protein (StAR), P450 side-chain cleavage enzyme (P450scc), and 17β-hydroxysteroid dehydrogenase (17β-HSD), which are essential for testosterone biosynthesis (Mavaddat et al., 2025). Increased StAR expression facilitates cholesterol transport into mitochondria, where P450scc catalyzes the formation of pregnenolone as a testosterone precursor (Martin & Touaibia, 2020). Similar mechanisms occur in the positive control group, where metformin enhances steroidogenic gene expression, thereby supporting testosterone production and improving spermatogenesis (Shpakov., 2021).

Tannins, another compound present in *Clitoria ternatea*, also contribute to improvements in spermatozoa quality in hyperglycemic rats. Tannins function as antioxidants and provide protective effects on spermatozoa. Physiologically, spermatozoa possess low levels of antioxidant enzymes, making them highly vulnerable to oxidative damage. Tannins enhance endogenous antioxidant activity, including SOD, catalase (CAT), and glutathione peroxidase (GSH-Px). Dai et al, (2020) reported that low-dose tannin administration (3 mg/mL) increased endogenous antioxidant levels and improved spermatozoa quality compared to both normal and high-dose groups. Enhanced endogenous antioxidant activity protects the sperm plasma membrane from lipid peroxidation, maintaining cellular integrity and increasing the proportion of viable spermatozoa. This mechanism explains the significant improvement in viability observed in group P3.

The use of herbal medicines remains largely limited to extracts, decoctions, or teas, resulting in suboptimal absorption of active compounds. This limitation is associated with the low bioavailability of *Clitoria ternatea* bioactive compounds, which tend to have poor water solubility, large molecular size, and limited permeability across lipid membranes (Hanutami & Budiman, 2017). To overcome these limitations, innovations in herbal drug delivery systems are required. One such approach is nanoherbal formulation. A commonly used preparation method is ionic gelation, which involves crosslinking polyelectrolytes and multivalent ions, such as sodium alginate and calcium chloride ($CaCl_2$). Nano-sized formulations offer several advantages, including more efficient delivery to target cells, increased surface area for absorption, improved solubility, and enhanced bioavailability in the small intestine (Achrifa & Suyatno, 2024). The nanoherbal *Clitoria ternatea* particles in this study had a size of 350.5 nm, which falls within the effective range for drug delivery. Nanoherbal particle sizes typically range from 50–500 nm, a range that facilitates cellular uptake and effective therapeutic delivery (Samudra et al., 2021).

Conclusion

Butterfly pea flower (*Clitoria ternatea*) nanoherbal formulation significantly improved spermatozoa quality, including concentration, motility, and viability, in hyperglycemic rats. These findings indicate that the nanoherbal formulation enhances the effectiveness of active compound delivery and bioavailability. From a biotechnological perspective, this study contributes to the development of nanotechnology-based herbal formulations as a potential approach for ameliorating reproductive disorders associated with hyperglycemic conditions. However, further studies are required to elucidate the underlying molecular mechanisms, evaluate hormonal parameters, and conduct preclinical as well as clinical studies.

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Competing of interest: The authors declare that there are no financial or personal relationships that could be construed as a potential conflict of interest.

Author's contributions: Komala Amelia Putri: conceived and designed the study, conducted the experiments, performed data analysis, and wrote the manuscript. **Wulan Christijanti**: provided guidance and scientific input during the design of the experiment, research implementation, data analysis, and manuscript preparation. **Aditya Marianti**: contributed to the experimental design through conceptual input and academic discussion. All authors approved the final version of the manuscript.

Generative AI: Not applicable

Data availability: The raw data supporting the conclusions of this article, including spermatozoa concentration, motility, and viability data obtained from experimental animals, will be made available by the authors, without undue reservation, to any qualified researcher.

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