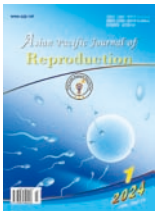

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Pro-fertility effect of *Ficus carica* fruit extract in streptozotocin-induced male rats

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ABSTRACT

Objective: To explore the impact of *Ficus carica* fruit aqueous extract on fertility parameters in streptozotocin (STZ)-induced male rats.

Methods: Twenty-four male Sprague-Dawley rats were divided into four different groups. All groups except a normal control group were induced with 50 mg/kg of streptozotocin (STZ) intravenously to induce diabetes. A positive control group was treated with an antidiabetic drug, metformin (500 mg/kg) whereas a negative control group remained untreated throughout the experiment. Meanwhile, another diabetic rat group received treatment with 400 mg/kg of aqueous *Ficus carica* fruit extract. Rats in the treatment group were administered *Ficus carica* fruit aqueous extract daily through force-feeding *via* oral gavage for a 21-day period. Assessments included the sperm quality (count, motility and morphology), histology of the testes, serum testosterone and fasting blood glucose (FBG) level.

Results: The FBG level of the *Ficus carica*-treated rats exhibited a significant decrease compared to the negative control group (P<0.05). Sperm quality analysis also indicated that the aqueous *Ficus carica* extract had significant positive effects on sperm count and motility (P<0.05). The histology of the testes in *Ficus carica*-treated rats revealed an improved cell arrangement in the germinal cell layer. Furthermore, serum testosterone level showed an increment in the *Ficus carica* treatment group in comparison to the negative control group.

Conclusions: Our findings provide compelling evidence for the pro-fertility and anti-hyperglycemic properties of aqueous *Ficus carica* fruit extract in diabetic-induced male rats.

KEYWORDS: *Ficus carica* fruit; Male fertility parameter; Diabetes mellitus; Anti-hyperglicemic

1. Introduction

Complications in male fertility are on the rise both locally and globally, assessed through sperm quantity and quality. Sengupta *et al* highlighted a 57% decline in average sperm concentration among men from 1980 to 2015[1]. Diabetes mellitus is identified as a major factor influencing sperm quality and disrupting spermatogenesis[2]. Male diabetic patients often report sexual dysfunctions, including a reduction in testosterone levels, loss of libido and erectile dysfunction, potentially leading to infertility if untreated[3]. These issues are further complicated by spermatogenesis disorders resulting from hypothalamic-pituitary gonadal dysfunction[4]. The global increase in diabetes and male infertility has prompted the development of various treatments involving chemical drugs such as glibenclamide, metformin for anti-diabetes and laboratory procedures such as intracytoplasmic injection (ICSI), *in vitro*

Significance

Prior knowledge indicates that diabetes can adversely affect reproductive health in males, impacting blood glucose levels and fertility parameters. This study, using *Ficus carica* fruit extract in diabetic-induced male rats, reveals promising results. It demonstrates that *Ficus carica* extract reduces blood glucose levels significantly and positively influences sperm quality, testes histology and serum testosterone levels. These findings suggest the potential of *Ficus carica* extract in countering diabetes-induced reproductive health issues, marking a significant advancement in exploring natural remedies for diabetic-related fertility complications.

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fertilization for fertility and Viagra as libido enhancement. However, these approaches have certain drawbacks, including reduction in sperm quality and the high costs associated with assisted reproductive technology.

In response to these challenges, the use of herbs as an alternative treatment is gaining popularity due to their potential to address infertility and other common diseases based on their known medicinal properties. Scientifically proven herbs with pro-fertility and anti-hypergicemic properties include *Gynura procumbens*[5], *Moringa oleifera*[6] and *Ficus carica*[7], all of which were reported in our previous study. *Ficus carica* is rich in nutrients and acts as a potent antioxidant, containing vitamins, zinc and flavonoids that have the potential to enhance male fertility while reducing blood glucose levels. Ain *et al* have demonstrated that *Ficus carica* significantly increases insulin levels in males, possibly contributing to increased fertility due to elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH)[8]. *Ficus carica* is exceptionally rich in phytochemicals found in both its leaves and fruits. However, only a limited number of studies have explored the potential of *Ficus carica* in treating both diabetes and male infertility and the mode of action of *Ficus carica* fruits in addressing both complications remains largely unexplored. Therefore, this study is conducted to investigate the potential of *Ficus carica* fruit aqueous extract in improving the fertility parameters of streptozotocin (STZ)-induced male rats. It is hypothesized that the administration of *Ficus carica* fruit aqueous extract will exhibit anti-diabetic effects in STZ-induced male rats by regulating blood glucose levels. Consequently, this extract may positively impact the fertility parameters of these rats by mitigating the adverse effects of diabetes on reproductive function, potentially through its antioxidant and metabolic regulatory actions.

2. Materials and methods

2.1. Preparations of aqueous Ficus carica fruit extract

The *Ficus carica* fruits were collected from Universiti Kebangsaan Malaysia Research Station at Kuala Pilah, Negeri Sembilan and were authenticated by the Herbarium of Faculty of Science and Technology, Universiti Kebangsaan Malaysia with voucher specimen number 40389. About 2.5 kg of *Ficus carica* dried fruits were ground to fine powder and distilled for 6 h at 60 ℃. The extract was filtered through Whatman paper No. 3 and stored at -80 ℃ for three days before it was freeze-dried. The final product of freeze-dried was used for the treatment group at 400 mg/kg.

2.2. Experimental design

A total of 24 male Sprague-Dawley rats, aged between 9 and 10 weeks and weighing approximately 300-350 g, were sourced from the Animal House at Universiti Kebangsaan Malaysia. The rats were divided equally (n=6) into four groups composed of three control groups: normal (healthy rats), negative (untreated diabetic rats) and positive (metformin-treated diabetic rats) and a treatment group (*Ficus carica* extract-treated diabetic rats). Prior to the commencement of treatment, all rats underwent a seven-day acclimatization period and were induced with diabetes through STZ injection, excluding the normal control group. Throughout the experimental period, the rats were provided with a standard pellet diet and had access to water *ad libitum*. Rats in the positive control group received treatment with 500 mg/kg of metformin, while those in the treatment group were treated with 400 mg/kg of aqueous *Ficus carica* fruit extract[9,10]. The treatment was administered through force-feeding *via* oral gavage once daily for a duration of 21 days. All rats were housed at a temperature of 28 ℃ under a 12-hour light/dark cycle and were strictly maintained according to established protocols.

2.3. Induction of diabetes and fasting blood glucose (FBG) test

All animal groups except normal control were injected with intravenous injection of STZ to induce diabetes at a dosage of 50 mg/kg, which was prepared earlier by dissolving in 0.1 M citrate buffer, pH 4.2. Rats with blood glucose level of 13 mmol/L and above were considered diabetic. The final FBG level of all rats was measured after 21 days of oral treatment using Glucometer Accucheck Active® Roche Diagnostic (Canada). The body weight of each animal was measured before and after treatment.

2.4. Sperm quality analysis

On the 22nd day following the measurement of FBG, all rats were sacrificed by cervical dislocation. The cauda epididymis was excised and placed in pre-warmed 15 mL Biggers-Whitten-Whittingham (BWW) medium. The composition of the sperm medium included 94.5 mM NaCl, 4.8 mM KCl, 1.7 mM $CaCl_2$, 1.2 mM $KHPO_4$, 1.2 mM $MgPO_4.7H_2O$, 25.1 mM $NaHCO_3$, 5.5 mM glucose, 0.25 mM sodium pyruvate, 21.6 mM sodium lactate and 10 mM penicillin-streptomycin, adjusted to a pH of 7.4 and supplemented with bovine serum albumin (BSA). The epididymal tissue was meticulously minced within the medium to facilitate the dispersion of sperm into the solution. Subsequently, the sperm suspension underwent incubation in a 5% carbon dioxide incubator at 37 ℃ for 30 min to capacitate the sperm. The assessment of sperm quality, encompassing count, motility and morphology, was conducted following the protocols outlined in the laboratory manual[11].

2.5. Testosterone hormone analysis

Blood samples were collected through cardiac puncture for the estimation of serum testosterone levels. The analysis was conducted

using a commercial kit following the manufacturer's instructions (Testosterone kit by Cayman Chemical, Michigan, USA).

2.6. Determination of testes histology

The left testis of each rat was excised and fixed overnight in Bouin's solution prior to dehydration in a series of graded alcohols and xylene. Next, the testes were embedded in paraffin wax and sectioned at 5 μm thickness. The slides were stained with hematoxylin and eosin (H & E) before observation was made under a light microscope at magnifications of 200× and 400× (Digital Microscope Carl Zeiss, Germany).

2.7. Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics version 22 software (SPSS Inc., USA). Data were analysed by *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's test for *post hoc* analysis. The results were presented as mean and standard deviation (mean±SD), while the value of $P<0.05$ was considered statistically significant.

2.8. Ethics statement

This study was approved by the Animal Ethics Committee of Faculty of Medicine, Universiti Kebangsaan Malaysia (FST/2017/MAHANEM/29 –MAC./833-MAC.-2017-MAC.-2019).

3. Results

3.1. Body weight and FBG level

The induction of diabetes resulted in a slight decrease in body weight in both the untreated negative control group and the group treated with metformin ($P>0.05$) compared to their pre-treatment weight. Interestingly, *Ficus carica*-treated rats showed no significant changes in body weight before and after diabetes induction. Conversely, the normal control group exhibited an increase in weight gain (Figure 1).

The study on the anti-hyperglycemic effects of *Ficus carica* involved conducting the FBG test on the 22nd day before sacrificing the rats for further analysis. Throughout the experiment, healthy rats in the normal control group maintained a stable FBG level ranging from (4.4±0.10) mmol/L to (4.9±0.20) mmol/L. As illustrated in Figure 2, the diabetic rats in the negative group exhibited no improvement in FBG levels but worsened with time as their FBG level increased from (19.86±2.73) mmol/L initially to (23.66±0.88) mmol/L after 21 days. Meanwhile, *Ficus carica* extract at 400 mg/kg dosage had successfully reduced the FBG level of the treated rats from (16.78±3.44) mmol/L to (10.08±1.67) mmol/L. The FBG level of the positive group, which was treated with metformin, also decreased greatly from (24.42±3.27) mmol/L to (13.22±0.81) mmol/L after 21 days of treatment. In the assessment of blood glucose control, both *Ficus carica* fruit extract and metformin exhibited statistically significant reductions in FBG levels compared to the negative control group ($P<0.001$). Moreover, the difference in blood glucose levels between the *Ficus carica*-treated and metformin-treated groups was found to be non-significant ($P=0.182$), suggesting that *Ficus carica* extract is as effective as metformin in controlling blood glucose levels in STZ-induced rats.

3.2. Sperm quality analysis

In Table 1, it is evident that rats in the normal control group exhibited a total sperm count of $(88.60\pm6.85)\times10^6$. The induction of diabetes has reduced the sperm count to $(2.80\pm1.36)\times10^6$ for untreated diabetic rats (the negative control group). Metformin-treated diabetic rats showed a slight improvement with an average sperm count of $(14.00\pm3.65)\times10^6$ compared to the negative group. Notably, the *Ficus carica*-treated group displayed the highest sperm count at $(103.60\pm10.77)\times10^6$, indicating a significant increase compared to the negative control group ($P=0.019$).

Considering sperm morphology, healthy rats in the normal control group had a normal sperm morphology percentage of (33.74±3.57)% (Table 1). The *Ficus carica*-treated rats exhibited a significantly higher percentage of normal sperm morphology at (41.46±6.48)% ($P=0.021$), whereas metformin-treated rats showed a slight improvement at (21.36±5.18)% ($P=0.469$) compared to the negative control rats, which displayed the lowest percentage of normal sperm morphology at (5.05±1.43)%.

The analysis of sperm motility involved categorizing the grade of sperm movement into three categories—progressive, non-

Table 1. Sperm quality assessment.

Parameters	Normal	Negative	Positive	*Ficus carica*
Sperm count, $\times10^6$	88.60 ± 6.85^{ab}	2.80 ± 1.36^{a}	14.00 ± 3.65^{a}	103.6 ± 10.77^{b}
Normal sperm morphology, %	33.74 ± 3.57^{ab}	5.05 ± 1.43^{a}	21.36 ± 5.18^{ab}	41.46 ± 6.48^{b}
Sperm motility, %				
Progresive	40.10 ± 4.22^{a}	6.80 ± 4.16^{b}	48.60 ± 5.68^{a}	56.75 ± 3.93^{a}
Non-progresive	14.44 ± 4.55^{ab}	0.00 ± 0.00^{a}	5.06 ± 1.27^{a}	32.98 ± 3.06^{b}
Immotile	25.52 ± 4.94^{ab}	53.32 ± 6.98^{a}	6.38 ± 1.44^{b}	10.29 ± 1.79^{b}

Normal: normal control group; negative control group: untreated STZ-induced rats; positive control group: metformin-treated STZ-induced rats; *Ficus carica* group: STZ-induced rats treated with 400 mg/kg of *Ficus carica* fruit extract. Different letters denote significant difference between various treatment groups ($P<0.05$).

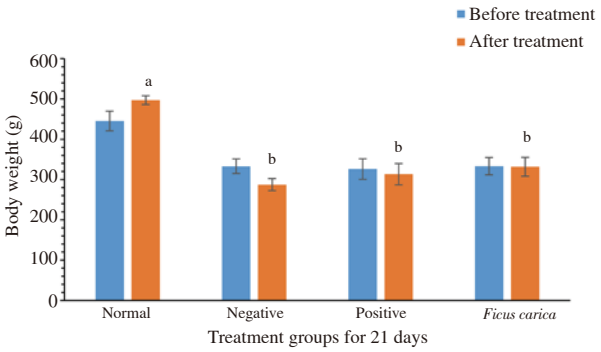

Figure 1. Body weight of the normal control group, negative control group (untreated STZ-induced rats), positive control group (metformin-treated STZ-induced rats) and *Ficus carica* group (STZ-induced rats treated with 400 mg/kg of aqueous *Ficus carica* fruit extract). Different letters indicate significant difference between various treatment groups (P<0.05).

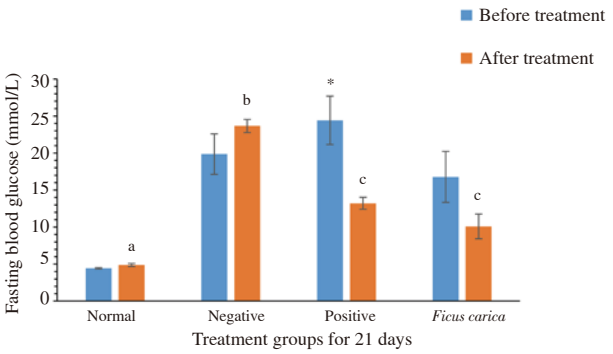

Figure 2. Fasting blood glucose level (FBG) of the normal control group, negative control group (untreated STZ-induced rats), positive control group (metformin-treated STZ-induced rats) and *Ficus carica* group (STZ-induced rats treated with 400 mg/kg of aqueous *Ficus carica* fruit extract). *denotes significant difference between before and after treatment groups (P<0.05). Different letters indicate significant difference between various treatment groups (P<0.05).

progressive and immotile[11]. Undoubtedly, the impact of diabetes on sperm quality was evident, with the percentage of progressive sperms for untreated diabetic rats (negative control) significantly dropping to (6.80±4.16)% (P=0.035) after 21 days of treatment, compared to the normal control group that exhibited progressive sperms at (40.10±4.22)% (Table 1). Treatment with *Ficus carica* extract significantly increased the percentage of progressive sperms in diabetic rats to (56.75± 3.93)% (P=0.017), a noteworthy improvement compared to the negative control group. Across all three parameters of the sperm quality test (count, morphology and motility), aqueous *Ficus carica* fruit extract demonstrated exceptional results, improving all aspects studied when compared to both the negative and positive control groups. This emphasizes the potential of *Ficus carica* extract in ameliorating diabetes-induced complications and underscores its effectiveness in promoting overall sperm quality.

3.3. Testosterone level

Figure 3 demonstrates a significant decrease in testosterone levels in the untreated diabetic rat group (0.39±0.032) ng/mL compared to the normal group (1.81±0.06) ng/mL (P=0.007). On the other hand, the administration of *Ficus carica* showed a notable effect in improving testosterone level (1.62±0.05) ng/mL, bringing it closer to the level observed in the normal group.

3.4. Testes histology analysis

In the histological examination of the testis, qualitative analysis was conducted on cross-sections of seminiferous tubules, focusing on the density of spermatogonia cells, primary spermatocytes, spermatids and spermatozoa. The observation also included the density of Sertoli cells, which fill and occupy the space within the inner seminiferous tubules and Leydig cells, which are situated between the seminiferous tubules. Figure 4A illustrates the histological features of normal control rats, revealing a healthy testicular structure devoid of damage. The epithelial germ cells in

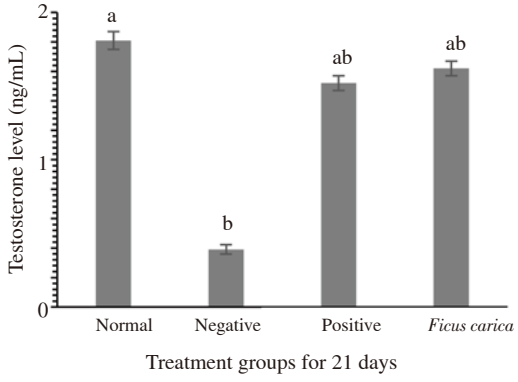

Figure 3. The effect of *Ficus carica* treatment on testosterone level in diabetic male rats. Different letters indicate significant difference between various treatment groups (P<0.05).

these rats exhibited a dense and well-arranged pattern, with a lumen filled with spermatozoa and no apparent impairment to the testicular cells. The impact of diabetes on the testis of rats was clear in the negative control group (Figure 4B), where damages to the germinal epithelium and inhibited spermatogenesis resulted in a reduction of spermatids within the tubules. The seminiferous tubules in this group appeared disorganized spermatogenic cell layer, with several vacuole appeared in most of the tubule sections. The cross-section of the negative control group was notably the most compromised among all groups, aligning with expectations. The *Ficus carica*-treated group exhibited an improved arrangement of the germinal cell layer, characterized by closely packed of tubules, in contrast to both the positive (Figure 4C) and negative control groups (Figure 4B). The tubules of *Ficus carica*-treated rats displayed a smaller lumen compared to the control groups, resulting in increased spermatogenic density with more sperms observed in the luminal space of tubule seminiferous. Importantly, no inhibition of spermatogenesis was noted and Leydig and Sertoli cells reappeared (Figure 4D).

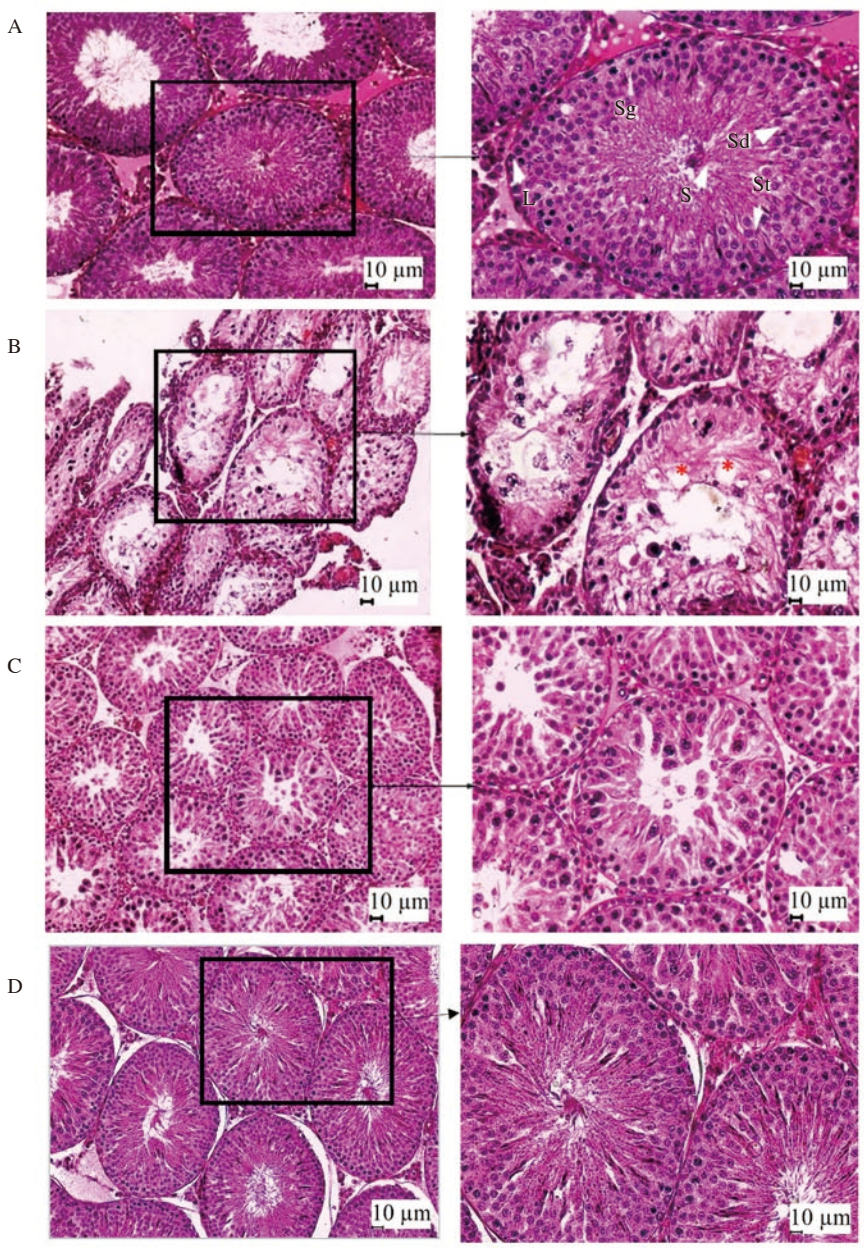

Figure 4. Photomicrograph of histological section of testis in the normal control group (A), negative control group (B), positive control group (C), and *Ficus carica*-treated group (D). Spermatogonia (Sg), primary spermatocytes (St), spermatids (Sd), spermatozoa (S), Leydig cell (L) in the normal control group. Red * indicates the presence of vacuole. Magnification: 200× and 400×. H & E staining.

4. Discussion

The mechanism by which *Ficus carica* enhances male fertility remains relatively unexplored, despite numerous studies investigating the impact of *Ficus carica* fruit on male reproductive health. This research has established the effectiveness of *Ficus carica* extract in reducing blood glucose levels and its potential to ameliorate diabetes in rats during a 21-day treatment period. Previous studies have consistently highlighted the anti-hyperglycemic properties of both *Ficus carica* fruits and leaves[12]. The *Ficus carica* fruits are rich in phenolic acids, flavonoids and anthocyanins, all of which exhibit potent antioxidant properties[13]. According to Mopuri *et al* the anti-hyperglycemic activity of *Ficus carica* fruit extracts can be attributed to their high antioxidant capacity[14].

Furthermore, *Ficus carica* fruits have demonstrated superior *in vitro* antioxidant capacity compared to *Ficus carica* leaves and stembark, resulting in enhanced anti-hyperglycemic and anti-obesogenic effects by inhibiting relevant enzyme activities. The *Ficus carica* extract acts as an inhibitor of carbohydrate digestive enzymes, including α-amylase and α-glucosidase, thus reducing blood glucose levels by impeding carbohydrate breakdown[15]. Therefore, the anti-diabetic effects of *Ficus carica* extract stem not only from its high antioxidant capacity but also from its ability to inhibit relevant digestive enzymes, representing a multifaceted approach to managing diabetes.

Our study has also revealed that the decrease in normal sperm

morphology is associated with the unimproved diabetic condition over the 21-day period. These findings indicate that *Ficus carica* treatment not only surpasses metformin in ameliorating diabetes in rats after 21 days of treatment but also significantly improves sperm morphology, underscoring its potential in mitigating the adverse effects of diabetes on sperm quality. This improvement contributes to an overall enhancement in sperm quality in our study. It is important to note that an increase in testosterone levels can indeed enhance sperm quality[5,16]. These results are consistent with previous findings which concluded that sperm quality parameters are affected in cases of diabetes[17,18]. Additionally, Kamaruzaman *et al* found that diabetic rats had lower serum testosterone levels than healthy rats, suggesting that the decline in testosterone levels may be attributed to glucose or its metabolites or defective gonadotropins[5].

Oxidative stress emerges as a significant factor contributing to infertility in diabetes-induced rats, particularly in the presence of hyperglycemia[19]. In this context, the diabetic condition triggers the excessive production of reactive oxygen species (ROS), a type of free radical found in the blood, leading to oxidative stress. Aiken and Baker further emphasized that the imbalance between increasing ROS and a deficiency of antioxidants in the body can result in a decline in sperm motility and damage to the sperm cell membrane[20]. Given that oxidative stress is a major contributor to testicular damage and a reduction in sperm quality[21], one proposed mechanism is that *Ficus carica* extract enhances fertility parameters by reducing ROS levels through anti-apoptotic effects[22]. Pérez *et al* also supported this mechanism, concluding that *Ficus carica* treatment successfully reduced oxidative stress in STZ-induced diabetic rats[23]. The ability of *Ficus carica* to reduce oxidative stress is believed to be the result of the cumulative effect of bioactive compounds in *Ficus carica* fruits with high antioxidant properties[24]. A previous high-performance liquid chromatography (HPLC) study conducted on *Ficus carica* fruit extract revealed the presence of phenolic compounds such as chromotropic acid, quercetin, gallic acid, caffeic acid, vanillic acid, syringic acid and m-coumaric acid[25]. Among these compounds, quercetin is recognized as a strong antioxidant that successfully reduces oxidative stress in STZ-induced diabetic models[26]. Furthermore, studies on quercetin have shown that its administration improves sperm quality parameters in STZ-induced diabetic rats, with these ameliorative effects attributed to its ability to reduce sperm oxidative stress and inflammation[27]. Additionally, Haredy *et al* suggested that *Ficus carica* contains saponins with a mode of action similar to testosterone, which can improve semen quality[16]. This proposition is supported by the findings of a phytochemical qualitative analysis conducted by Palaniyappan *et al* who discovered numerous bioactive components in *Ficus carica* fruits, including flavonoids, tannins and saponins[28]. In the same study, *Ficus carica*-treated rats exhibited improved libido and better copulatory sexual behavior towards female rats. Therefore, our study aligns with Palaniyappan *et al* suggesting that *Ficus carica* extract can increase testosterone availability for testicles, potentially contributing to an enhancement in reproductive quality[28]. Thus, the phytoconstituents of *Ficus carica* fruits are believed to be responsible for both its anti-hyperglycemic and pro-fertility effects on STZ-induced rats.

Additionally, our histological findings are in line with those reported by Ghanbari *et al* and Ghosh *et al*[29,30]. The latter conducted histological analysis on STZ-induced rats' testicles and identified impaired germinal epithelium structure, reduced spermatids and diminished numbers of testicular cells, including Leydig and Sertoli cells[30]. Similarly, Ghanbari *et al* reported diabetes-induced atrophy, a reduced diameter of seminiferous tubules and impaired spermatogenesis[29]. They suggested that the lack of insulin in diabetic rats might lead to decreased levels of testosterone, FSH and LH, impacting endocrine function and spermatogenesis. This proposition is supported by previous studies which demonstrated a reduction in androgenic hormones in diabetic rats through immunoradiometric assays[5,31].

Our observations also indicated that *Ficus carica* fruit extract produced superior results compared to metformin in restoring testicular structure and spermatogenesis. The previously conducted studies have reported that testicular damage, resulting from oxidative stress, was effectively restored through *Ficus carica* extract treatment[16,32]. An increase in antioxidant enzymes, testosterone levels and semen quality, along with a reduction in testicular oxidative stress markers were also observed in rats treated with *Ficus carica*[16]. The ability of *Ficus carica* to reduce ROS, a main factor in testicular recovery, is believed to stem from the phytochemical content of the fruits, including phenolic compounds, phytosterols, organic acids, anthocyanin composition, triterpenoids and coumarins[33].

These consistent findings across different studies underscore the multifaceted impact of diabetes on male reproductive health, encompassing hormonal imbalances, oxidative stress and consequential effects on sperm quality parameters. This collective evidence supports the notion that addressing these aspects is crucial for developing effective strategies to manage fertility complications in individuals with diabetes. However, this study has limitations, notably the relatively modest sample size of 24 male rats distributed across four groups. The 21-day timeframe might limit assessing enduring impacts of *Ficus carica* fruit extract on blood glucose levels and fertility parameters. Additionally, as the study was exclusively conducted on induced diabetic rats, extrapolating these findings to naturally occurring diabetes in human subjects requires further investigation.

In conclusion, the empirical findings presented in this study offer significant insights into the effects of aqueous *Ficus carica* fruit extract on reducing blood glucose levels, increasing testosterone level and improving overall sperm quality in STZ-induced rats over a 21-day treatment period. Notably, the extract appeared to

enhance the arrangement of cells at the germinal cell layer and increase spermatogenesis in diabetic-induced rats. These results substantiate the efficacy of aqueous *Ficus carica* fruit extract as both an anti-hyperglycemic and pro-fertility agent, warranting further investigation to elucidate the specific mechanisms by which *Ficus carica* fruit improves reproductive parameters in diabetic models.

Conflict of interest statement

The authors declare no conflicts of interest to disclose.

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This study received no extramural funding.

Authors' contributions

Mat Noor Mahanem conceived and designed the study. Subramaniam Puvaratnesh conducted the experiments and collected the data. Shamsusah Nadia A performed the statistical analysis. Subramaniam Puvaratnesh and AbuBakar Umarqayum wrote the manuscript. Mat Noor Mahanem and Shamsusah Nadia A critically reviewed and edited the manuscript. All authors contributed to the interpretation of results and approved the final version for submission.

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