

Hygrophila auriculata seed extract mitigates infertility caused by streptozotocin-induced diabetes in Wistar albino rat model

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Abstract

The present study investigates the effect of an ethanolic extract of *Hygrophila auriculata* seeds on the male reproductive organ weights, testicular lipid peroxidation and antioxidant potential, testosterone levels, and effects on sperm quality parameters in a streptozotocin (STZ)-induced diabetic male rat model, a widely used experimental model of diabetes-associated infertility. The extract was administered orally to STZ-induced diabetic rats to evaluate its effect on reproductive parameters in terms of evaluating sperm count, motility, viability, morphology and chromatin dispersal assay along with organ weight of male reproductive organs and serum testosterone levels. Antioxidant activity in testicular extract was assessed by measuring lipid peroxidation, glutathione peroxidase, superoxide dismutase, reduced glutathione and catalase assays. The results revealed that although the male reproductive organ weights decreased significantly in diabetic and treatment groups as compared to the control, the semen quality parameters such as sperm count, motility and viability improved significantly ($p < 0.05$) in the treatment groups. The *H. auriculata* seed extract significantly ($p < 0.05$) reduced lipid peroxidation in the testicular tissue as compared to the untreated diabetic controls. *H. auriculata* seed extract was not observed to have ameliorative potential against sperm DNA damage. The ethanolic extract of *H. auriculata* seed significantly ($p < 0.05$) improved serum testosterone concentration as compared to the control and diabetic rat model groups implying an androgenic effect of the extract. The study provides evidence that the ethanolic extract of *H. auriculata* seeds protects testicular integrity and sperm quality by reducing lipid peroxidation levels in a diabetic rat model.

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels caused by either insulin resistance or insufficiency. As of 2021, 537 million people worldwide suffer from diabetes, making it a global public health concern (Sun et al. 2022). While the global burden of diabetes is a well-documented human crisis, it is also becoming significant in pet animals especially dogs and cats. Studies indicate that dogs frequently suffer from an insulin deficiency similar to Type 1 diabetes (Abhijith et al. 2025), whereas cats often develop an insulin-resistant form akin to Type 2 diabetes (Samaha et al. 2019). Two major forms of diabetes are – Type 1 diabetes (T1D), an autoimmune response that destroy beta cells of pancreas that synthesize insulin, and Type 2 diabetes (T2D), which is usually associated with lifestyle choices and obesity. It is commonly associated with systemic complications, such as nephropathy, neuropathy, cardiovascular disorders, and cancer. Both types have negative effects on a number of organs, including the reproductive system, which ultimately results in infertility (Graziani et al. 2024). In large monogastric animals, chronic hyperglycaemia leads to systemic complications including neuropathy and cardiovascular dysfunction (Wolf et al. 2026). In all mammals, disruption of the hypothalamic-

pituitary-gonadal (HPG) axis results in reduced sperm concentration, impaired motility, and morphological defects.

The pathophysiology of diabetes not only makes glycaemic control more difficult but also impacts oxidative stress and chronic inflammation. An imbalance in reactive oxygen species (ROS) causes cellular damage by lipid peroxidation, protein oxidation, and DNA damage, which collectively deteriorate male reproductive functions (Luo et al. 2025). In humans, comprehending the relationship between diabetes and reproductive health is crucial for improving fertility management strategies in couples suffering from diabetes. About 10 to 15% of couples worldwide who are of reproductive age suffer from infertility, a complicated multifactorial medical issue that causes severe emotional and psychological suffering (American Diabetes Association 2019). Infertility is influenced by a variety of factors, including lifestyle choices, anatomical anomalies, and hormone imbalances (Sethiya et al. 2018). Diabetes has been demonstrated to have a detrimental effect on hormonal balance and reproductive health, resulting in reduced sperm concentration, impaired motility, and abnormal morphology in men, and polycystic ovarian syndrome (PCOS), tubal factors, and uterine abnormalities in women (Maresch et al. 2018; Zegers-Hochschild et al. 2017). Recent research has demonstrated a strong correlation between diabetes and infertility with diabetes linked to erectile dysfunction and

poor semen quality in men, and hormonal abnormalities with impaired ovarian function in women (La Vignera et al. 2012). Hyperglycaemia is not just a metabolic condition but also the primary source of oxidative stress since it produces ROS. Mammalian sperm are especially vulnerable to oxidative damage due to the high concentration of polyunsaturated fatty acids in the plasma membrane and the lack of antioxidant enzymes in the cytoplasm. The diabetes model produced by STZ accurately mimics the long-term oxidative environment observed in human diabetic patients. This model, called the oxidative reproductive model, helps to evaluate how the blood-testis barrier is broken down and DNA fragmentation occurs as a result of hyperglycaemic-induced LPO. This paradigm can be used specifically to evaluate *H. auriculata*, which acts as a mitochondrial antioxidant that may neutralize ROS at the testicular level and maintain sperm integrity during metabolic disease (Huang et al. 2024).

In this context, an aquatic plant, *H. auriculata*, has long been utilized in Ayurvedic medicine due to its many health advantages, such as its anti-inflammatory, antibacterial, and antioxidant properties. Notably, *H. auriculata* minimizes oxidative damage in cells by enhancing key protective pathways. Recent research has acknowledged the potential of medicinal plants in the management of oxidative stress-related illnesses, diabetes, and infertility (Anusha et al. 2013). Therefore, the objective of the present study was to analyse the potential beneficial effects of *H. auriculata* seed extract on testicular oxidative levels and sperm quality attributes in streptozotocin-induced diabetic male rat models of infertility.

2. Materials and Methods

2.1 Experimental animals and study design

Adult male Wistar albino rats (n = 30) were randomly divided into five experimental groups (n = 6) as detailed in Table 1. Diabetes was induced in rats with STZ, followed by subsequent treatment with *H. auriculata* seed ethanolic extract and standard drug (gliclazide), crushed and mixed with distilled water, administered orally for a period of 60 days. In the present study, STZ was selected to induce diabetes due to its preferential cytotoxicity against pancreatic beta-cells and in addition, STZ mimics the oxidative stress induced by hyperglycaemia in clinical diabetic subfertility cases (Alzain et al. 2021). Gliclazide (10 mg/kg) was chosen as the reference drug due to its demonstrated antioxidant capacity to protect sperm functional attributes from oxidative

degradation in addition to its insulinotropic effects (Oztaş et al. 2019). At the end of the experimental period, the animals were euthanized and blood samples were collected.

2.2 Extract preparation

H. auriculata seeds were collected from the area in and around Hosur, Krishnagiri district, Tamil Nadu, India. The collection site is geographically situated at approximately 12.7408° N latitude and 77.8253° E longitude, with an elevation of 880 meters above sea level. This region is characterized by its unique semi-arid climate and plateau topography, which influences the phytochemical profile of the species. The *H. auriculata* seeds were botanically authenticated by Dr. Mutheeswaran, Scientist, Xavier Research foundation, Palayankottai. Reg. no: XCH- 40594. The seeds were thoroughly washed with distilled water to remove dust, debris, any foreign matter and the cleaned seeds were shade-dry for an appropriate duration until a constant weight is achieved. Direct sunlight or high heat exposure was avoided, as this was a cold extraction procedure. The dried seeds were ground into a coarse powder using an electric grinder (particle size of 30-40 mesh) to increase the surface area for efficient extraction. Ethanol extraction was carried out in the present study due to its effectiveness in recovering a wide variety of bioactive secondary metabolites, such as sterols and flavonoids, which are recognized for their pro-fertility and antioxidant qualities. This ensures the extraction of important androgenic components like stigmasterol and β-sitosterol, which are recognized as potential phytochemical compounds in *H. auriculata* (Ghosh and Mallick 2020; Gokilavani and Arunkumar 2026). Additionally, the ethanol reduces the possibility of solvent-induced toxicity in the experimental animals.

A known quantity (500 g) of the seed powder was taken in a clean, stoppered glass container (maceration vessel) and the ethanol was added in a suitable ratio (e.g.1:5 to 1:10 w/v. Then the mixture was sealed and cold macerated at room temperature (20-25°C) for a period of 3 to 7 days. The sample in the container was mixed frequently at least twice daily to ensure solvent penetration and high extraction efficiency (Dhanalakshmi et al. 2020). After maceration, the mixture was strained to separate the liquid extract from the solid residue and then filtered through Whatman No. 1 filter paper. The marc was pressed to recover any remaining trapped solvent. The filtrate was concentrated using a rotary vacuum evaporator (rotavapor) under reduced pressure at a low temperature typically below 40-45°C to remove the ethanol. This gentle evaporation prevented the degradation of thermolabile bioactive compounds and the process was continued until a viscous crude extract was obtained and further dried in a vacuum desiccator. Finally, the dried crude extract was weighed and the percentage yield based on the initial weight of the seed powder was calculated as follows:

Yield % = Weight of dried extract / Weight of dried seed powder x 100

The dried extract was stored in an airtight, amber-coloured container at refrigerated conditions until use. The extract was typically suspended in 0.5% (w/v) normal saline for oral administration.

2.3 Assessment of testicular oxidative stress markers

The experimental rats were euthanized and dissected in order to obtain various organs. For assessing oxidative stress markers in testis, immediately after collection the samples were washed with phosphate buffer saline and used for estimation of antioxidant enzyme

Table 1. Experimental design			
Groups	Sample size	Group specification	Intervention
Control	6	Vehicle control	Only saline (0.5 ml/kg)
STZ	6	Diabetic control	Streptozotocin (STZ 60 mg/kg, i.p)
STZ + STD	6	Standard control	STZ (60 mg/kg, i.p) + 10 mg/kg (p.o) of gliclazide
STZ + HA 100 mg	6	Test -1 (low dose)	STZ (60 mg/kg, i.p) + EEHA low dose 100 mg/kg (p.o)
STZ + HA 200 mg	6	Test -2 (high dose)	STZ (60 mg/kg, i.p) + EEHA high dose 200 mg/kg (p.o)
STZ: Streptozotocin; STD: Standard (Gliclazide); HA: <i>Hygrophilla auriculata</i> ; EEHA: Ethanolic extract of <i>Hygrophilla auriculata</i> ; i.p: Intraperitoneal; p.o: per os			
The animals were fed for 60 days and euthanized at the end of the feeding trial			

assessment.

2.3.1 Glutathione peroxidase (GPx)

The reaction mixture consisted of 0.2 ml each of Ethylenediamine-tetraacetic Acid (EDTA), sodium azide, and H_2O_2 , 0.4 ml of phosphate buffer, and 0.1 ml of testicular homogenate. After incubation at 37°C for 10 minutes, the reaction was arrested by addition of 0.5 ml of trichloroacetic acid and the tubes were centrifuged at 2000 rpm. 0.5 ml of supernatant was taken into a tube followed by the addition of 4 ml of disodium hydrogen phosphate and 0.5 ml 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). The absorbance was read at 420 nm immediately and the activity of GPx was expressed as moles of glutathione oxidized/min/mg protein (Graziani et al. 2024).

2.3.2 Catalase (CAT)

The assay measures the decrease in absorbance of H_2O_2 following its decomposition by catalase into water and oxygen. The phosphate buffer (0.01 M, pH 7.0), H_2O_2 (0.2 M), and a 5% potassium dichromate/acetic acid solution (1:3 ratio) was added to 1 ml of tissue homogenate. The enzymatic reaction was initiated by adding the homogenate to the buffered substrate at room temperature. At 1-minute intervals, 1 ml of the mixture was taken and added to 2 ml of the dichromate/acetic acid reagent. The acidic dichromate reagent served to stop the reaction by denaturing the enzyme. The resulting mixture was incubated for 30 minutes for the formation of the chromic acetate complex and absorbance was measured spectrophotometrically at 570 nm. The CAT activity was expressed as μmoles of H_2O_2 consumed/min/mg protein (Cheung et al. 2015).

2.3.3 Superoxide dismutase (SOD)

SOD activity was estimated based on inhibition of adrenaline auto-oxidation. The reaction mixture consisted of 0.4 ml adrenaline, 1.5 ml carbonate buffer (pH 10.2), 0.5 ml of 0.1 Mm EDTA, and 0.5 ml supernatant tissue homogenate. The absorbance was measured at 480 nm. Epinephrine was added just before taking the optical density. The activity of SOD was expressed as units/min/mg protein. One unit of the enzyme is defined as the amount of enzyme, which inhibits the rate of adrenaline auto oxidation by 50% (Santi et al. 2021).

2.3.4 Reduced glutathione assay (GSH)

GSH was estimated based on the established procedure (Al-Khawaldeh et al. 2024). Briefly, after adding 1 ml of 5% TCA to 250 μl of tissue homogenate in a 2 ml Eppendorf tube, the mixture was centrifuged at 3000 g for 10 minutes at room temperature. The supernatant was collected (250 μl) and 1.5 ml of 0.2 M phosphate buffer was added and thoroughly mixed. To this mixture, 250 μl of 0.6 mM Ellman's reagent (DTNB solution) was added and the absorbance at 412 nm was measured in ten minutes. Glutathione reduction solution (1 mg/ml) was used to determine the amount of GSH in the tissue homogenates. Glutathione content was expressed in milligrams of protein.

2.3.5 Lipid peroxidation (LPO)

To 1 ml of tissue homogenate, 0.2 ml of 4% (w/v) sodium dodecyl sulfate, 1.5 ml of 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5), and 1.5 ml of 0.8% thiobarbituric acid (TBA, pH 7.4) were added and the mixture was heated to 85°C for one hour in a hot water bath. Then the sample mixture was centrifuged at 1200 g for 10 minutes. The supernatant was collected and the optical density was measured at 532 nm against a reagent blank. Using 1,1,3,3-tetra-ethoxypropane as the

standard, the concentration was reported as nano moles per mg of protein (Ohkawa et al. 1979).

2.4 Sperm collection for DNA fragmentation assay

At the end of the experimental period, male Wistar albino rats were euthanized, and the cauda epididymis was immediately harvested and cleared of adipose tissue. To assess sperm parameters, the cauda was placed in 2 ml of pre-warmed (37°C) phosphate buffered saline (pH 7.4). The tissue was finely minced to allow the spermatozoa to diffuse into the medium. After 10 minutes of incubation, the resulting sperm suspension was used for the determination of sperm count, motility, and morphological abnormalities according to the standard protocol (Vernet et al. 2001).

2.4.1 Sperm DNA fragmentation assay

DNA fragmentation was carried out as per the procedure described in the kit (Sperma frag rinse reagent kit, Vitrotech Private Limited, Chennai, Tamil Nadu, India). The concentration of semen sample was assessed and diluted with culture medium to 5-10 M/ml. Solution A (0.08 N HCl) was added to denature the sperm. The agarose was melted at 90°C in sperm chroma warmer 1 for 5 minutes. Then the agarose was transferred to the sperm chroma warmer 2 maintained at 37°C for 5 minutes (Vitro-Warm, Vitrotech Pvt Ltd, India). The semen sample (25 microlitres) was added to the agarose and mixed well. The sperm cell suspension was immediately transferred onto the pretreated slides and coverslip was applied. After incubation at 4°C for 5 minutes, the cover glass was removed by sliding it off gently. After a further 7 minutes of incubation, the slide were kept horizontally in lysis solution for 25 minutes. Then, the slides were dipped in distilled water for 5 minutes. Subsequently, the slides were placed in a series of ethanol solutions - 70% ethanol (2 min), followed by 90% ethanol (2 min), and finally in 100% ethanol (2 min). The slides were allowed to dry at room temperature. The Wright's stain (solution C) and Giemsa stain (solution D) (1: 1), were mixed and stained the slides for 15-20 minutes. Then the stain was decanted and slides were washed with distilled water and dried at room temperature. The slides were examined under bright field microscope using 40x objective and the spermatozoa were graded. If the staining was too strong, the slide could be destained by rinsing gently in distilled water or 10% ethanol. At least 200 spermatozoa were counted per slide and the cells were classified based on morphology. The Sperm DFI was calculated based on the presence of halos of different sizes surrounding sperm heads as small halo, without halo, and degraded categories. The presence of other cells such as immature germinal cells, WBC or epithelial cells was also counted.

2.5 Serum testosterone levels

Serum testosterone levels were quantified using an automated Competitive Chemiluminescence Immunoassay (CLIA) (Maglumi series, Vitrotech Pvt Ltd, India). The serum samples were separated by centrifugation from blood and stored at $2 - 8^\circ\text{C}$. The testosterone calibrators, quality control materials were equilibrated to room temperature before use. The assay used ready-to-use magnetic microbeads coated with anti-testosterone antibody and an acridinium ester-labelled testosterone conjugate. The labelled testosterone competes with the unlabelled testosterone (from the sample) for limited binding sites on the magnetic beads. The cuvette was incubated at 37°C for a specified period (10 to 30 minutes) to allow the competitive binding equilibrium to be reached. After incubation, magnetic separation was performed and the cuvette was washed multiple times

with a wash buffer to remove all unbound testosterone. The analyser introduced the starter reagents (oxidiser are HNO_3 & H_2O_2 as Starter 1 and catalyst of NaOH as Starter 2) to initiate the chemical reaction, which generated chemiluminescence measured by a photomultiplier tube (Cheung et al. 2015). The testosterone concentration was calculated using a 2-point calibration master curve. The results are expressed in ng/ml. The signal was recorded as Relative Light Units (RLU).

2.6 Histopathological examination

Histopathology of the reproductive organs including testis, prostate, epididymis, seminiferous tubules, and vas deferens was carried out. After washing, the tissues were cut into 3 to 5 mm thick and fixed in 10% neutral buffered formalin for 24-48 h at room temperature. The organs were dehydrated in an alcohol series (30%, 50%, 70%, 90%, and 100%) and cleared in xylene. Then the sections were cleaned in tap water. The cleaned tissues were embedded in molten paraffin at 58°C, sectioned at 5µ thickness, and stained with Harris hematoxylin and eosin. The stained sections were mounted using DPX mountant, covered with coverslip without air bubbles, dried, and examined under a light microscope (40x) for observing histopathological changes (Jalil et al. 2017).

2.7 Statistical analysis

Percentage data related to sperm functional parameters were arcsine transformed prior to statistical analysis. The analysis was carried out using SPSS software by one-way ANOVA followed by Dunnett's post hoc comparison. The vales were presented as mean ± standard error of the mean (SEM) and the differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Organ weight

Testis: The current study results revealed a non-significant decrease in testicular organ weight among the control, diabetic model, and treatment groups (Table 2).

Cauda epididymis and Vas Deferens: The diabetic model group showed a significant decrease in cauda epididymis and vas deferens weights compared to the control group. In comparison to the STZ group, all treatment groups displayed lower ($p < 0.05$) vas deferens weights.

Seminal Vesicle: The STZ group had substantially lower ($p < 0.01$) seminal vesicle weight than the control group. However, seminal vesicle weight was significantly lower ($p < 0.001$) in all treatment groups compared to the STZ group.

Prostate: No statistically significant variations were observed in the weight of the prostate between the groups.

3.2 Sperm functional attributes

3.2.1 Count

The STZ group had a significantly ($p < 0.05$) lower sperm concentration than the control group. Sperm count increased significantly in the STZ + STD group, even surpassing the control group concentration. The STZ + HA100 group sperm count increased significantly compared to the STZ group, but remained significantly lower than the control group. However, the sperm count in the STZ + HA200 group improved significantly compared to the STZ group and reached the concentration level of the control group (Table 3).

3.2.2 Motility

The STZ group had significantly ($p < 0.05$) lower sperm motility compared to the control group. The motility in the STZ + STD group further decreased significantly ($p < 0.05$) compared to the STZ group. The motility of the STZ + HA100 group improved significantly ($p < 0.05$) compared to the STZ group and sperm motility significantly improved in the STZ + HA200 group to the level of the control group.

3.2.3 Viability

The STZ group exhibited significantly ($p < 0.05$) lower sperm viability than the control group. The viability in the STZ + STD group further decreased significantly ($p < 0.05$) compared to the STZ group. Comparing the STZ + HA100 group to the STZ group, viability increased significantly. Sperm viability was significantly restored in the STZ + HA200 group to the level of the control group.

3.2.4 Abnormalities

The sperm abnormalities were significantly ($p < 0.05$) higher in the STZ group than the control group. The STZ + STD group revealed significantly reduced abnormalities compared to the STZ group and the values are comparable to control group. Though STZ+HA100 group had higher sperm abnormalities compared to the control, STZ+HA200 group had significantly lower abnormalities than the STZ and STZ+HA100 groups.

3.3 Serum testosterone

The serum testosterone level decreased significantly in the STZ group compared to control, which further increased non-significantly in the STZ + STD group compared to STZ group (Fig. 1). Treatment with *H. auriculata* significantly ($p < 0.05$) improved serum testosterone concentration compared to the STZ +STD treatment and STZ alone groups implying androgenic effect of the seed extract.

3.4 Testicular oxidative stress

Activities of superoxide dismutase, catalase, and glutathione peroxidase did not differ significantly among the groups. The GSH

Table 2. Organ weights (g) of Wistar rats

Organ	Control	STZ	STZ + STD	STZ + HA100	STZ + HA200
Testis	1.423±0.030	0.985±0.211	1.156±0.235	1.103±0.148	1.335±0.030
Vas Deferens	0.615±0.193	0.271±0.017	0.201±0.043	0.214±0.036	0.212±0.040
Epididymis	0.475±0.036 ^a	0.308±0.034 ^a	0.260±0.047 ^b	0.224±0.046 ^b	0.268±0.028 ^b
Seminal vesicle	1.395±0.114 ^a	0.793±0.140 ^a	0.355±0.074 ^b	0.504±0.105 ^b	0.592±0.099 ^b
Prostate	0.633±0.053 ^a	0.443±0.042 ^a	0.240±0.078 ^b	0.296±0.051 ^b	0.386±0.035 ^b

STZ: Streptozotocin; STD: Standard (Gliclazide); HA: *Hygrophilla auriculata*
Means bearing different superscripts (a, b) within the row differ significantly ($p < 0.05$)

Table 3. Sperm quality attributes of Wistar rats

Quality attributes	Control	STZ	STZ + STD	STZ + HA100	STZ + HA200
Count (millions/ml)	77.2±0.50 ^a	43.2±1.06 ^b	74.9±0.53 ^a	63.4±0.49 ^a	84.6±0.29 ^a
Motility (%)	79±0.20 ^a	60.5±0.35 ^b	53.7±2.22 ^b	69.7±2.4 ^a	86.7±0.62 ^a
Viability (%)	84.6±0.36 ^a	66.3±0.22 ^b	52.7±0.83 ^b	75.3±1.04 ^a	86.5±1.05 ^a
Abnormality (%)	21.8±0.85 ^b	37.3±0.66 ^a	24.1±0.96 ^b	30.2±0.17 ^a	16.1±0.25 ^b

STZ: Streptozotocin; STD: Standard (Gliclazide); HA: *Hygrophilla auriculata*
Means bearing different superscripts (a, b) within the row differ significantly (p<0.05)

levels in the STZ group was significantly higher than the control group. The LPO levels in the STZ group were significantly (P<0.05) higher compared to the control group, suggesting enhanced lipid peroxidation (Table 4).

40x, characterized by atrophied seminiferous tubules, disorganized cell layers, and a decline in the number of mature spermatozoa. In the STZ + STD group, partial restoration of testicular morphology compared to the STZ group can be seen, but to a lesser extent when compared to the

Table 4. Antioxidant status of Wistar rats. The units for values of SOD (units/mg protein), CAT (μmole of H₂O₂ consumed/min/mg protein), GPx (μmol NADPH oxidized/min/mg protein), LPO (nmol of MDA/mg protein)

Antioxidant attributes	Control	STZ	STZ + STD	STZ + HA100	STZ + HA200
SOD	0.413±0.005	0.409±0.045	0.329±0.001	0.332±0.004	0.349±0.009
CAT	0.226±0.003	0.270±0.001	0.224±0.002	0.241±0.004	0.214±0.005
GPx	0.269±0.007	0.288±0.005	0.260±0.005	0.280±0.002	0.265±0.018
GSH	0.172±0.003 ^b	0.202±0.006 ^a	0.169±0.002 ^b	0.160±0.003 ^b	0.171±0.004 ^b
LPO	0.070±0.023 ^b	0.141±0.007 ^a	0.121±0.003 ^a	0.088±0.006 ^b	0.057±0.003 ^b

STZ: Streptozotocin; STD: Standard (Gliclazide); HA: *Hygrophilla auriculata*
SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxide; GSH: Reduced glutathione; LPO: Lipid peroxidation
Means bearing different superscripts (a, b) within the row differ significantly (p<0.05)

3.5 Sperm DNA integrity

All four groups with diabetes and treatment exhibited a high percentage of sperm with DNA fragmentation as the highest DFI of 66% was observed in STZ+ HA200, followed by 64% in STZ+ STD, 63% in STZ, and 62% in STZ+ HA100 (Table 5).

3.6 Histopathological findings

The control group exhibited a well-organized complement of spermatogenic cells, with typical testicular architecture, including distinct seminiferous tubules (Fig. 2). Healthy, mature spermatozoa were abundantly seen in the epididymis. In the STZ group, notable pathological alterations in the testicular architecture were observed in

STZ+HA100 and STZ+HA200 groups. In *H. auriculata* supplemented groups, a dose-dependent protective effect was observed in the testicular tissue, with increased number of spermatogenic cells, higher mature sperm cell count, and restored cellular organization in the seminiferous tubules.

4. Discussion

The study offers valuable insights into the effects of STZ-induced diabetes and subsequent treatment with *H. auriculata* seed extract on reproductive organ morphology, sperm quality, hormonal status, oxidative stress, and testicular histology of male Wistar rats (Ghasemi et al. 2014). Diabetic metabolic disturbances are known for their adverse effects on the reproductive physiology through alterations in energy

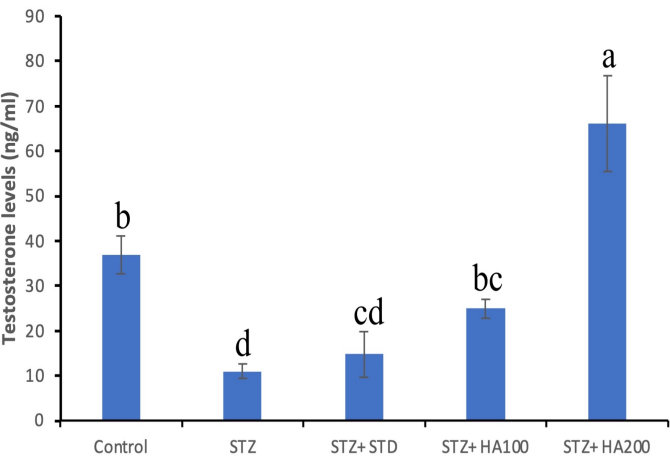


Fig. 1. Testosterone levels (ng/ml) of Wistar rats

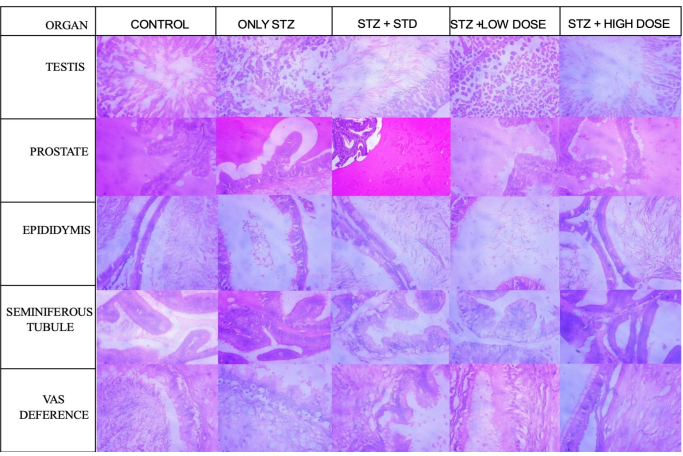


Fig. 2. Histology of Wistar rats

Table 5. DNA fragmentation index of analysed sperm using sperm chromatin dispersal test in Wistar rats

Antioxidant attributes	Status	Control	STZ	STZ + STD	STZ + HA100	STZ + HA200
Big halo (%)	Without fragmentation	45	17	29	16	12
Medium halo (%)	Without fragmentation	15	20	7	20	22
Small halo (%)	With fragmentation	35	50	64	44	56
Without halo (%)	With fragmentation	6	9	0	12	2
Degraded (%)	With fragmentation	2	4	0	6	8
DNA fragmentation index		43%	63%	64%	62%	66%

STZ: Streptozotocin; STD: Standard (Gliclazide); HA: *Hygrophilla auriculata*
 SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxide; GSH: Reduced glutathione; LPO: Lipid peroxidation
 Means bearing different superscripts (a, b) within the row differ significantly (p<0.05)

metabolism, endocrine regulation, and oxidative balance (Dena et al. 2024). Importantly, Though the testis weight was not significantly affected by STZ, vas deferens, epididymis, seminal vesicle and prostate was affected by diabetes. The prostate, seminal vesicles, caudal epididymis, and vas deferens are extremely androgen-dependent organs. Although the seed extract was anticipated to exert a protective effect by increasing or normalizing reproductive organ weights toward control levels, the present study observes such restoration only in the testis. The exact reason for this is not clear. The reasons could be the dosage used and the duration of the study. The *H. auriculata* is a widely consumed traditional herb with a reported LD₅₀ > 2000 mg/kg (OECD 423), justifying the safety of the 100 and 200 mg/kg doses used (Vijayakumar et al. 2006). This implies that the duration of study needs to be longer to observe beneficial effect of the standard medication and the extract from *H. auriculata* in overcoming the atrophy caused by diabetes in these organs. It is also to be noted that the treatment compound may also potentially intensifies catabolic state of diabetes (Sethiya et al. 2018). Even if the weight of the testes remains constant, histopathological evaluation and sperm parameter measurements are essential for assessing their functional integrity. The STZ-induced diabetes resulted in considerable atrophy in a number of androgen-dependent reproductive cells, but the effects of the standard medication and the seed extract from *H. auriculata* on reversal of negative effects were also observed (Martins et al. 2021).

The results from the study unequivocally reveal that male infertility stems from serious impairments to sperm properties caused by STZ-induced hyperglycaemia in Wistar albino rats. Sperm count, motility, and viability were decreased in the diabetic model group. Chronic diabetes frequently affects sperm quality due to hormonal imbalances, increased testicular apoptosis, and oxidative stress. The sperm quality parameters were improved upon the administration of *H. auriculata* seed extract. The high dose of the extract (200 mg/kg) was successful in reducing sperm abnormalities and enhancing sperm count, motility, and viability compared to the control. This implies that *H. auriculata* mitigates the negative effect of diabetes on semen quality. The extract probably may improve the microenvironment of the testes, and promote healthy spermatogenesis by lowering oxidative stress and inflammation (Rastogi et al. 2014). In-depth molecular and biochemical studies are required to fully understand the mechanisms behind these findings and to determine the true protective effects of the *H. auriculata* seed extract in diabetes-induced male infertility (Barkabi-Zanjani et al. 2020).

Sperm DNA fragmentation is a crucial sign of male reproductive failure, and numerous research have shown a direct correlation

between diabetes and sperm DNA fragmentation. However, the treatment could not reduce the DNA fragmentation index in diabetic rats suggesting that the dose could not completely overcome the oxidative stress and the dose needs to be optimized for further improving fertility in males (Roy et al. 2023). Depending on the dosage and length of the treatment, some anti-diabetic drugs may have variable or even negative effects on particular aspects of male reproductive parameters (Santi et al. 2018). This implies that although synthetic hypoglycaemic medications work well to control blood sugar, they might not be enough to lessen the localized oxidative stress and inflammatory cascade that STZ causes in the testicular microenvironment.

Diabetes significantly increased LPO in testicular tissue, and resulted in oxidative damage to cell membranes and enhanced LPO levels in the system. However, the levels of SOD, CAT, and GPx levels did not differ among the groups. The extract appears to exert its effects through direct free-radical scavenging rather than the upregulation of endogenous antioxidant enzyme systems. The high phenolic and flavonoid content provide an immediate defence against ROS in the testicular microenvironment. This protects the sperm membrane by reducing LPO and thereby improves motility even when the endogenous enzymatic system (SOD/GPx) has not yet fully recovered from the STZ-induced insult in the present study. The bioactive sterols present in *H. auriculata* may function as competitive inhibitors of oxidative damage, akin to the free-radical scavenging ring present in the gliclazide molecule, whereas STZ causes DNA alkylation and subsequent testicular oxidative stress. The present study showed a significant increase in GSH levels in the STZ-induced diabetic group compared with the control. This may be attributed to an adaptive antioxidant response during the early phase of diabetes, where oxidative stress activates cellular defence mechanisms including increased glutathione synthesis. Activation of anti-oxidant pathways and tissue-specific protective mechanisms in the testis may also contribute to this elevation. However, prolonged hyperglycaemia is expected to deplete GSH due to excessive oxidative stress. The extract's strong antioxidants, such as phytosterols (β -sitosterol) reported in an earlier study (Gokilavani and Arunkumar 2026) may neutralize testicular ROS. Earlier studies also revealed that 12 compounds and among these β -sitosterol, stigmasterol and N-decanoic acid compounds were found to have higher antidiabetic activity and androgenic (Manigandan et al. 2021; Gokilavani and Arunkumar 2026). The positive effect observed in the present study could be due to the presence of these chemicals.

In the present study, a reduced testosterone levels were observed in

the diabetic model and standard treatment group. This is similar to the observation made earlier that the reduced testosterone production is known to result from the direct impact of diabetes on Leydig cell function and impairment of the HPG axis (Kotian et al. 2019). In the treatment groups, the improved testosterone positively impacts sperm quality and improve male fertility. Overall, by elevating testosterone levels and reducing lipid peroxidation, *H. auriculata* seed extract demonstrates significant potential in mitigating diabetes-induced male infertility. The lack of significant recovery in accessory sex organ weights in the plant extract treated groups suggests that the structural improvements in these tissues require a longer duration than the duration of the experiment conducted this study. Hence further studies are required to elucidate its precise mechanisms and to determine the optimal dosage and duration needed to achieve hormonal balance and complete recovery of reproductive function (Simon et al. 2017; Maresch et al. 2018).

5. Conclusions

The seed extract of *H. auriculata* at a dose of 200 mg/kg has protective and restorative effects on sperm functional characteristics in diabetic rats and may be used as a natural remedy to improve male fertility in diabetic conditions.

Declarations

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