Prayogik Rasayan

Evaluation of *Aloe vera (L.)* in Mitigating Diabetes-Induced Oxidative Stress Linked Testicular Dysfunction

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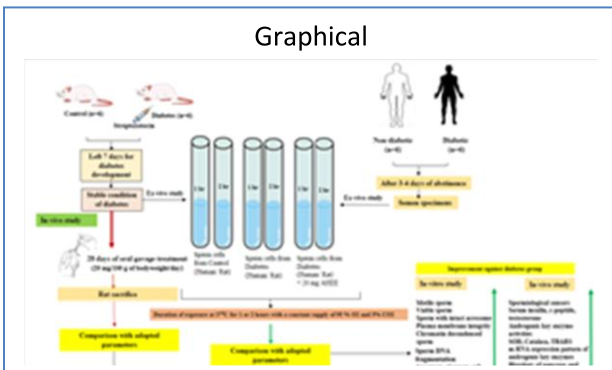
Abstract

Presently WHO giving emphasis on one-drug multi-target therapy for multi-faced diseases. Diabetes mellitus poses a significant global health challenge, exacerbating complications such as male infertility through hormonal disruptions, oxidative stress, and vascular complications. Epidemiological studies indicating that near about 50% diabetic male experience reproductive co-morbidity. This study investigates the efficacy of *Aloe vera (L.)* hydro-ethanol extract (AHEE) in alleviating diabetes-induced male infertility through rigorous in-vitro and in-vivo experimentation. Experimental diabetes was developed in male albino rats by intramuscular injection of streptozotocin (STZ) at the dose of 4 mg/ 100 g of somatic weight. Six diabetic and control (without STZ) rats were left untreated for 28 days, included in in-vitro study parallelly with in-vivo investigation. Experiment with six semen samples of diabetic and non-diabetic individuals were also considered in in-vitro study to observe the direct effect of AHEE on spermiological sensors. Treatments with AHEE was done for four weeks at 20 mg/100 g of somatic weight/day in rat, considered for in-vivo study with a control and diabetes group for comparison. In-vitro experiments demonstrated substantial improvements in motility, viability, acrosome integrity, decondesation status of sperm following exposure with AHEE in respect to untreated diabetes group (UDG) in both human and rat sperm. These rectifications were complemented by findings from the Comet and TUNEL assay, showing decreased DNA fragmentation in sperm cells of human and rat compared to UDG. Flow cytometry analysis with Annexin V corroborated these results by indicating reduced apoptotic sperm cells, indicative of improved cellular survival at post-exposure to AHEE. In-vivo investigations using diabetic rat models highlighted the *Aloe vera (L.)*'s efficacy, showing significant reductions in fasting blood glucose level alongside elevated serum insulin, C-peptide, and testosterone levels following treatment with AHEE than UDG. Spermiological analyses demonstrated enhanced motility, plasma membrane integrity, acrosomal status, decondensation status of sperm in *Aloe vera (L.)* treated diabetes rats, alongside histological observations revealing mitigated



degeneration of pancreas and testis against UDG.

Biochemical assays further revealed a diminution in oxidative stress markers and enhanced activities of antioxidant enzymes, suggesting *Aloe vera (L.)*'s ability to mitigate oxidative damage and inflammation associated with diabetes-induced male infertility. Gene expression studies using qRT-PCR elucidated the *Aloe vera (L.)*'s impact on androgenic key enzymes, revealing upregulated expression of 17β-hydroxy steroid dehydrogenase and Δ5, 3β- hydroxy steroid dehydrogenase genes in extract-treated group than UDG. Liquid chromatography-mass spectrometry profiling identified major six bioactive compounds in AHEE, potentially elucidating mechanisms underlying its therapeutic effects. These comprehensive findings focused that AHEE is a potent natural agent exhibiting remediation for managing diabetes-related testicular complications. The integration of traditional medicinal knowledge with contemporary scientific approaches offers promising avenues for developing effective and affordable treatments. Further exploration of the molecular mechanisms underlying *Aloe vera (L.)*'s therapeutic effects and its clinical implications in human subjects is warranted, highlighting its potential as a valuable therapeutic agent in male reproductive health and diabetes management

Keywords: Diabetes, Male infertility, *Aloe vera (L.),* Oxidative stress, Apoptosis, Spermiological sensor

1. Introduction

Diabetes mellitus, a complex metabolic syndrome caused by impaired insulin secretion, function, or both, has become a global epidemic. This surge is largely influenced by modern lifestyle choices and the rapid pace of technological advancements, necessitating innovative strategies to address and manage the condition effectively.[1] This condition disrupts the metabolism of proteins, lipids, and carbohydrates, leading to impaired glucose homeostasis.[2] While the incidence of

diabetes is raising worldwide, developing countries like India are experiencing a particularly sharp increase in this concern, posing significant health risks and societal disruption.[3] Among the myriad complications associated with diabetes, male infertility has emerged as a significant concern, necessitating focused research to elucidate the underlying mechanisms and potential interventions. Male infertility in the context of diabetes is a multifaceted issue, resulting from a combination of endocrine disruptions, oxidative stress, and vascular complications. Diabetes can lead to hormonal imbalances, such as reduced levels of testosterone and other androgens, which are crucial for spermatogenesis and overall male reproductive health. Additionally, oxidative stress induced by chronic hyperglycemia damages sperm DNA and impairs sperm motility and viability.[4] Vascular complications further contribute to erectile dysfunction, exacerbating the challenges associated with diabetes-induced infertility.[5]

For millennia, plants have been the cornerstone of traditional medical systems across various cultures, and their relevance persists even in modern healthcare frameworks.[6] Nearly 80% people of developing nations took traditional medicine to get relief from different health problems due to the efficacy, minimal adverse effects, acceptability and affordability of herbal medicines.[7] The World Health Organization (WHO) endorses the application of traditional herbal treatments for various ailments, including diabetes mellitus.[8] Among these medicinal plants, *Aloe vera (L.)* stands out for its extensive therapeutic properties, including anti-inflammatory, antioxidant, immune-boosting, anti-aging, and anticancer activities.[9] *Aloe vera (L.)*'s unique biochemical composition has also led to its widespread use in industrial applications. Recent research has highlighted the potential of *Aloe vera (L.)* extracts in managing diabetes and its complications through various mechanisms. Given this context, our study aims to explore the non-genonic and genomic effect of *Aloe vera (L.)* through in-vitro and in-vivo studies respectively. Specifically, we focused on addressing the often-overlooked issue of diabetes-induced male infertility. By examining the potential therapeutic effects of *Aloe vera (L.),* we hope to contribute to the development of effective, affordable treatments that leverage traditional medicinal knowledge in addressing modern health challenges.

2. Experimental

Plant extract preparation

The gel from the leaves of 2- to 3-year-old *Aloe vera (L.)* plants was collected during June and July. Five hundred milliliters of the gel was added in one liter of hydro-ethanol (40:60) and left for two days. The resulting solvent-gel mixture was condensed using rota evaporator and then lyophilized. The obtained lyophilized extract was kept at -80°C for future experimental use.[10]

Ethical approval

Before conducting the experiment, the proposed investigation plan and design received approval from the Institutional Ethics Committee (IEC), with the approval number VU/IAEC-I/DG-1/3-15/19, dated December 11, 2019. Consent forms, prepared in consultation with the 'Medical Officer', Vidyasagar University and sanctioned by the Human Ethics Committee of the Institution (IHEC). These consent forms were duly filled in for obtaining semen samples.

Diabetes animal model

Upon delivery from the authorized vendor, rats weighing 120 ± 10 g and aged two months were kept 10 days under controlled conditions of temperature (25 ± 2°C) and humidity (45-60%). to acclimatize with the environment. Diabetes was induced by a single intramuscular injection of Streptozotocin (STZ) at a dose of 40 mg/kg of body weight.[10] The control group received an injection of citrate buffer (pH 4.5) at a dose of 1 ml/kg of body weight to match the physical stress level of the diabetic group due to the injection process. On the seventh day after STZ injection, fasting blood glucose (BG) levels were measured from the lateral tail vein. Rats with fasting BG levels between 300 mg/dl and 350 mg/dl were adopted as diabetic model animals for this study. The diabetic condition was maintained for next 28 days. One of the diabetic group was subjected to treatment with AHEE.

Experimental design for in-vitro study

Human semen samples and epididymal lumen washed sperm samples of rat were collected and processed as per the standard method.[11] The samples were divided into the following groups: control, hydro-ethanol extract (HEE)-uncharged diabetes, and 20 mg HEE-charged diabetes. Each group was further subdivided based on the charging duration into two sub-groups: 1 hour and 2 hours of exposure of the said extract to sperm samples.

Control

In each test tube, 10 ml of Krebs Ringer Bicarbonate (KRB), pH 7.0 was mixed with 0.5 ml of sperm suspension from normoglycemic cum fertile rat or 0.5 ml of semen from normoglycemic cum fertile individual. The mixture was then supplied with a gas mixture for 1 or 2 hours, and the ex-vivo experiment was conducted.

HEE-Uncharged Diabetes

Each test tube contained 10 ml of KRB solution and 0.5 ml of epididymal washed sperm suspension from diabetic rat or 0.5 ml of semen from diabetic person. The solution was supplied with 30 bubbles of gas mixture per minute for either 1 or 2 hours.

HEE-Charged Diabetes

In each test tube, 10 ml of KRB solution was mixed with 0.5 ml of sperm suspension from diabetic rat or 0.5 ml of semen from diabetic person. The mixture was charged with 20 mg of HEE of *Aloe vera (L.)* and processed as previously described for either 1 or 2 hours.

After incubating all samples at 37°C for either 1 or 2 hours with a continuous supply of gas mixture (O_2 and CO_2 at a

ratio of 9.5:0.5) at the specified flow rate, spermiological analysis was promptly conducted after completion of the exposure period with said extract in the ex-vivo condition.

Experimental design for in-vivo study

Six rats were assigned to each group and the treatment regimen was as follows-

Vehicle-Treated Control Group (VTCG)
Normoglycemic rats (fasting BG 70-80 mg/dl) in this group were administered 5 ml of deionized water/kg body weight (BW) via oral route at 9 O' clock morning on an overnight empty stomach.

Vehicle-Treated Diabetes Group (VTDG)
Diabetic rats (fasting BG 300-350 mg/dl) in the VTDG were given 5 ml of deionized water /kg BW through oral route at 9 O' clock morning on an overnight empty stomach.

Hydro-Ethanol Extract-Treated Diabetes Group (HEETDG)
Diabetic rats in the HEETDG were subjected for treatment with 200 mg of HEE of *Aloe vera (L.)* dissolved in 5 ml deionized water/kg somatic weight per day, administered orally under the same conditions. Food was provided 2 hours post-treatment to avoid food-phytomolecule interactions. On the 29th day, all the rats were sacrificed. Blood was collected for serum parameters analysis. The cauda epididymis was dissected to assess the adopted spermiological sensors. Testis and pancreas were washed in saline and stored at –20°C for biochemical analysis, while the other testis was processed for histological observation after Bouin's fixation.

Spermiological sensors
Motility, viability, hypo-osmotic swelling (HOS), nuclear chromatin decondensation (NCD), and acrosome intactness status (AIS) of sperm were assessed following established protocols for each parameter.[11]

Serum insulin, c-peptide and testosterone
Enzyme-linked immune-sorbent assay was used for the serum levels of insulin, c-peptide and testosterone assessment as per standard methods.[10,12]

Oxidative stress markers
Activities of superoxide dismutase, catalase, and the level of thiobarbituric acid (TBARS) reactive substances were assessed following well established method.[13]

Annexin-V
Sperm with fragmented DNA were detected by staining with propidium iodide (PI) and fluorescein iso-thiocyanate (FITC)-labeled Annexin V (FLAV) and then recorded using a flow cytometer (FACS Calibur, Becton Dickinson, San Diego, CA, USA). For each sample, ten thousand spermatozoa were counted. Channel FL1 detected FLAV-positive cells, while Channel FL2 measured PI-stained sperm cells.[14]

Comet assay
The prepared slides were soaked in lysis buffer for an hour at 40°C in the dark, then rinsed with deionized water and placed in electrophoresis buffer for 30 minutes. Next, the slides were immersed in neutralizing buffer three times for 5 minutes each and stained with ethidium bromide. The comet tailed cells were considered for counting using a fluorescence microscope, and the results represented in terms of percentage of the total cell count.[15]

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay
For noting the apoptosis and DNA fragmentation of sperm, the TUNEL assay was conducted through flow cytometry. Sperm samples were fixed for 1 hour at room temperature using 4% paraformaldehyde as a fixative and after that the sperm samples were rinsed in phosphate-buffered saline (PBS). Permeabilization was achieved using Triton X-100 (0.1%) in sodium citrate (0.1%) for 2 minutes on ice. The samples were then incubated with the TUNEL reaction mixture, containing terminal deoxynucleotidyl transferase (TdT) and fluorescence-dUTP, at 37°C for 1 hour in the dark. Negative and positive controls were prepared. After incubation, the sperm cells in samples were washed using PBS and then resuspended in PBS. Flow cytometry was performed to detect fluorescence-labeled dUTP incorporated into DNA strand breaks, with TUNEL-positive cells exhibiting increased green fluorescence, indicative of DNA fragmentation.[14]

Genomic study
Initially, RNA was extracted from testicular tissues and used for cDNA biosynthesis. Using reagents from Roche Diagnostics, Germany, the expression level of 17β-HSD and Δ5, 3β-HSD genes were then assessed by the replication of DNA for fixed number of cycles.[16]

Histology
Bouin's fixed pancreatic and testicular tissues were embedded in paraffin, and finely sectioned using a Leica semi-automated microtome. After performing hematoxylin and eosin staining, the sections were subjected to microscopic scanning at 400x magnification.[16]

Liquid chromatography-mass spectrometry (LC-MS)
QuattroMicroTMAPI mass spectrometer (Waters, Milford, Massachusetts, USA) was used for LC-MS analysis by a standard method with slight modification.[17]

Statistical Analysis
Analysis of Variance (ANOVA) followed by Multiple Comparison Student's two-tail t-test was followed for observing the significance level about hypotesticular and spermiological activities of the extract.[18]

3. Result and discussion

Our study investigated the therapeutic efficacy of *Aloe vera (L.)* HEE in mitigating diabetes-induced male infertility through both ex-vivo and in-vivo assessments. The results exhibited the significant improvements in adopted spermiological and biochemical parameters, providing insights into the mechanisms underlying these effects. The dominance of oxidative stress over the body's antioxidative defense mechanisms is a critical factor in the progression of sub-fertility linked to diabetes. Addressing oxidative stress by enhancing antioxidative capacity could be a pivotal strategy for alleviating diabetes-related reproductive dysfunction. Postprandial hyperglycemia initiates the sorbitol-aldose reductase pathway, glycation of protein, and advanced glycation end products (AGEs) formation all of which are major contributors to oxidative stress and subsequent vascular complications, including sub-fertility or infertility.[19,20]

Elevated levels of reactive oxygen species (ROS) lead to a decrease in intracellular adenosine triphosphate (ATP) concentrations, impairing sperm motility that is directly linked to ATP hydrolysis for energy production.[21,22] In the in-vitro study, motile sperm percentage was significantly deteriorated with the progression of time in the HEE- uncharged diabetes group (HDG) of rat and human **(Table 1 and 2).**

Table 1 In-vitro effect of *Aloe vera (L.)* on spermiological sensors in rat

Parameters	Rat							
	0-hour		1 hour of incubation			2 hours of incubation		
	HEE unexposed control	HEE unexposed Diabetes	HEE unexposed control	HEE unexposed Diabetes	HEE exposed diabetes	HEE unexposed control	HEE unexposed Diabetes	HEE exposed diabetes
% of motile sperm	65.33 ± 0.75^{a}	46.35 ± 0.58^{b}	58.52 ± 0.91^{c}	30.61 ± 0.67^{d}	40.41 ± 0.63^{e}	46.46 ± 0.58^{f}	25.15 ± 0.50^{g}	39.65 ± 0.71^{e}
% of viable sperm	88.14 ± 0.51^{a}	44.20 ± 0.56^{b}	81.19 ± 0.61^{c}	35.32 ± 0.51^{d}	41.65 ± 0.57^{e}	77.24 ± 0.65^{f}	29.20 ± 0.61^{g}	40.33 ± 0.61^{e}
HOS (%)	67.33 ± 0.51^{a}	33.68 ± 1.63^{b}	56.23 ± 0.75^{c}	22.63 ± 0.56^{d}	27.73 ± 0.55^{e}	45.20 ± 0.54^{f}	18.25 ± 0.55^{g}	26.68 ± 0.78^{e}
NCD (%)	75.33 ± 0.51^{a}	63.91 ± 0.61^{b}	69.41 ± 0.50^{c}	56.13 ± 0.65^{d}	60.11 ± 0.56^{e}	66.17 ± 0.65^{f}	33.75 ± 0.55^{g}	59.89 ± 0.70^{e}
AIS (%)	61.25 ± 0.68^{a}	35.96 ± 0.60^{b}	54.35 ± 0.68^{c}	24.47 ± 0.56^{d}	31.56 ± 0.49^{e}	50.66 ± 0.45^{f}	20.95 ± 0.45^{g}	30.11 ± 0.97^{e}

Data were expressed as Mean ± SEM, n=6. ANOVA followed by Multiple Comparison Student's Two-tail t-test. Values with different superscripts (a-g) in each row differ from each other significantly, $p<0.05$.

Table 2 In-vitro effect of *Aloe vera (L.)* on spermiological sensors in human

Parameters	Human							
	0-hour		1 hour of incubation			2 hours of incubation		
	HEE unexposed control	HEE unexposed Diabetes	HEE unexposed control	HEE unexposed Diabetes	HEE exposed diabetes	HEE unexposed control	HEE unexposed Diabetes	HEE exposed diabetes
% of motile sperm	78.30 ± 3.23a	57.58 ± 2.33b	72.23 ± 6.16c	45.45 ± 2.79d	53.45 ± 0.60e	65.42 ± 0.65f	36.55 ± 0.68g	52.45 ± 0.93e
% of viable sperm	83.48 ± 1.67a	48.25 ± 0.93b	79.16 ± 1.45c	41.11 ± 0.48d	45.11 ± 0.37e	71.34 ± 0.47f	30.54 ± 1.33g	44.50 ± 0.65e
HOS (%)	68.30 ± 1.80a	44.67 ± 0.65b	60.25 ± 0.50c	34.50 ± 0.90d	39.45 ± 0.97e	55.15 ± 0.60f	28.55 ± 0.65g	38.63 ± 0.58e
NCD (%)	74.10 ± 5.05a	61.51 ± 3.15b	70.15 ± 1.95c	57.30 ± 0.80d	58.22 ± 0.52e	67.19 ± 0.35f	49.90 ± 0.68g	57.70 ± 0.65e
AIS (%)	70.55 ± 0.63a	45.12 ± 0.92b	61.50 ± 2.32c	34.44 ± 0.70d	41.93 ± 0.86e	58.30 ± 3.05f	24.59 ± 0.67g	40.42 ± 0.67e

Data were expressed as Mean ± SEM, n=6. ANOVA followed by Multiple Comparison Student's Two-tail t-test. Values with different superscripts (a-g) in each row differ from each other significantly, $p<0.05$.

The observed stabilization of sperm motility deterioration after co-incubation with *Aloe vera (L.)* is likely due to improved ATP synthesis.[10]

Excessive ROS and end product of lipid peroxidation are known to disrupt permeability and integrity of membrane, inhibit membrane-linked enzymes of sperm along with reduced sperm's capacity for capacitation, acrosomal action cum activity, and oocyte fusion, which are crucial processes for fertilization. Sperm viability, HOS and AIS tests, focused that significant deterioration in the plasma membrane structure integrity and acrosome activity of sperm in diabetes group **(Table 1 and 2).** The maintenance of plasma membrane integrity and acrosomal status of sperm treated with *Aloe vera (L.)* both in ex-vivo and in-vivo have been attributed by the reduction of lipid peroxidation, likely due to the ROS-scavenging properties of flavonoids and phenolic phytocompounds present in the extract, or the inhibition of lipoxygenases that catalyze these oxidative reactions.[23,24] Additionally, the increased percentage of sperm with intact acrosomes in the HEE-charged diabetes group (HCG), both in ex-vivo and in-vivo suggesting better fertilization potential.
Oxidative damage contribute to apoptosis and DNA fragmentation, activating pathways like p53, leading to cytochrome c release and caspase activation.[25] This caspase-cascade ultimately compromises sperm genomic integrity and fertilization potential. Similarly, feedback-feedforward process of apoptosis associated mitochondrial ROS can provoke fragmentation of sperm DNA. The TUNEL and Comet assay along with Annexin V assay further confirmed the in-vitro protective effects of said extract on integrity of sperm DNA **(Figure 1 and 2).**

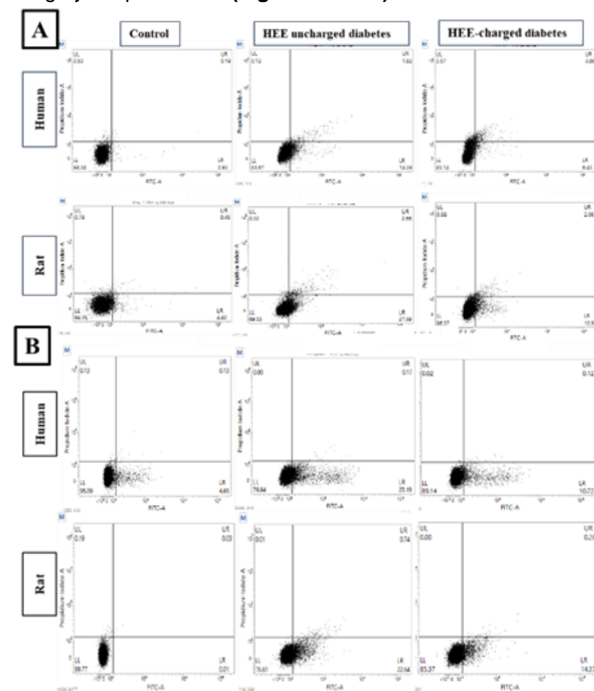


Figure 1. Assessment of apoptotic cell and DNA fragmentation of sperm through Annexin V and TUNEL assay respectively after 2 hours of in-vitro exposure. [A] Annexin V assay of human and rat sperm cells. [B] TUNEL assay of human and rat sperm cells

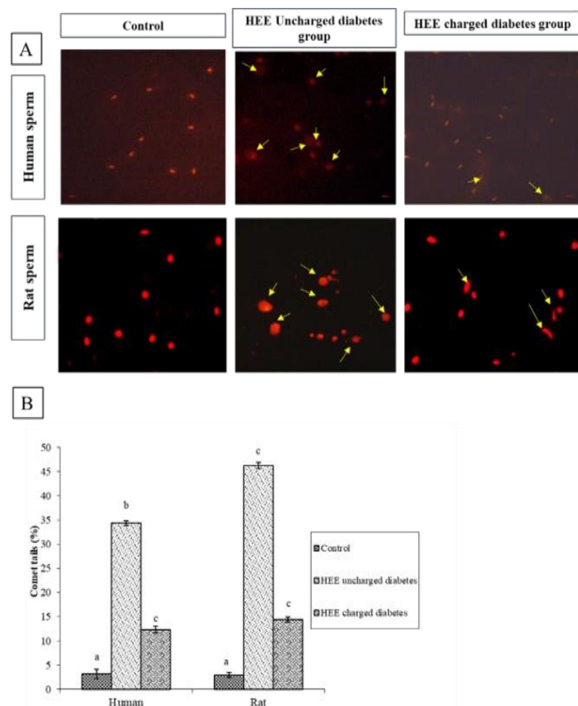


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Figure 2. DNA fragmentation assessment of sperm cell after in-vitro exposure by Comet assay in rat and human sample. [A] Microscopic view of Comet assay. [B] Percentage of Comet tails in treated groups.

TUNEL-positive cells and increased Comet tails, indicators of DNA fragmentation, whereas Annexin V positive sperm cells, indicators of apoptotic cells, were significantly reduced in the extract charged diabetes group in respect to uncharged diabetes group after 2 hours of incubation, suggesting that the extract mitigated the genotoxic effects of chronic hyperglycemia. The explanation of this rectification could be made by the phytomolecule(s), mainly the presence of flavonoid and phenol. The said nature of the phytomolecule (s) may able to cease lipid oxidation-peroxidation cascade reaction or can inhibit lipooxygenases and may be one of the mechanisms for such protective activity of *Aloe vera (L.)* on progressive loss of fertilizable spermatozoa in diabetic condition.[24,26] Another way may be, the phytomolecule (s) stabilizes the mitochondria and thus prevent electron leakage from electron transport chain, further reduces ROS generation, ensuring sperm energy and function.[27]

The in-vivo experiments supported these findings, with diabetic rats treated with HEE showing significant correction in fasting blood glucose levels in HEE-treated group (HTG), as deteriorated in untreated diabetic group (UDG). Streptozotocin selectively destroys pancreatic beta cells by causing DNA damage and oxidative stress, leading to necrosis or apoptosis. This destruction results in significantly reduced levels of insulin and C-peptide in the bloodstream.[28] Serum insulin and C-peptide levels were significantly elevated in *Aloe vera (L.)* treated group than UDG, indicating improved pancreatic function, further strengthen the result of fasting blood glucose level **(Figure 3).**

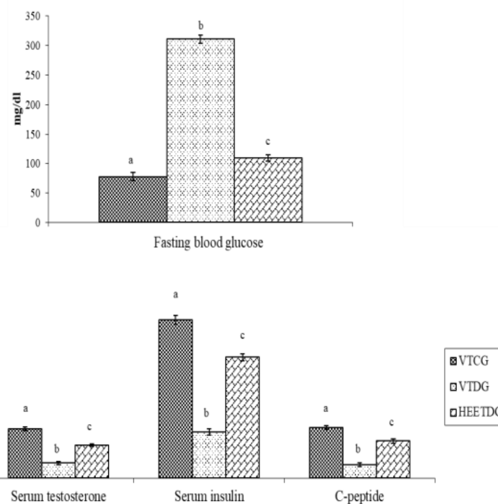

Figure 3. Correction of fasting blood glucose, serum testosterone, insulin and C-peptide levels after 28 days of oral treatment with *Aloe vera (L.).* Statistical tool 'Analysis of Variance' followed by Multiple Comparison Student's Two tail t-test was used. Each column represents Mean ± SEM, sample size (n) = 6. Column with different superscripts (a-c) differ from one another significantly, p<0.05

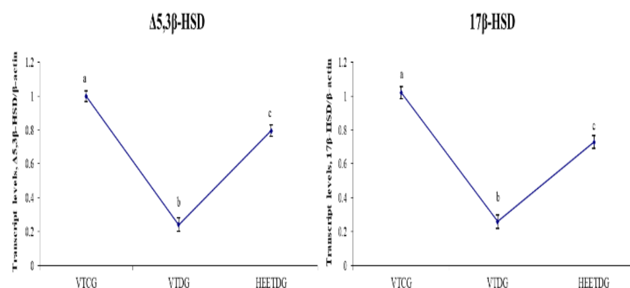

Figure 4. Improvement in mRNA expression pattern of androgenic key enzyme by *Aloe vera (L.)* treatment. Each point of the line diagram expressing mean ± SEM (n = 6). ANOVA followed by "Multiple comparison student's Two-tail t-test". Each point with different superscripts (a-c) differs from each other significantly, p<0.05.

Testosterone level, which was notably lower in diabetic rats, supported by reduced mRNA expression pattern and kinetics of key regulatory enzymes of androgen biosynthesis, was restored to near-normal levels in the HTG, suggesting enhanced endocrine function **(Figure 4 and 5).**[16] This was further supported by improved histoarchitecture of the pancreas may resulted in restoration of insulin's role in Leydig cell function. This restoration is significant because it activates the PI3K/Akt pathway, a critical signaling route that facilitates glucose uptake into cells. This pathway not only ensures efficient glucose metabolism but also plays a vital role in androgenesis. It achieves this by activating key testicular steroidogenic enzymes and important transcription factors such as SF-1 (Steroidogenic Factor 1) and LRH-1 (Liver Receptor Homolog-1), thereby enhancing the production of androgens.[10] **(Figure 6).**

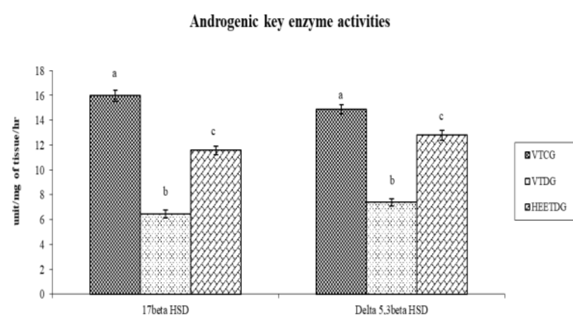

Figure 5. Remedial effect of *Aloe vera (L.)* on androgen synthesis regulatory enzyme kinetics. Statistical tool 'Analysis of Variance' followed by Multiple Comparison Student's Two tail t-test was used. Each column represents Mean ± SEM, sample size (n) = 6. Column with different superscripts (a-c) differ from one another significantly, p<0.05

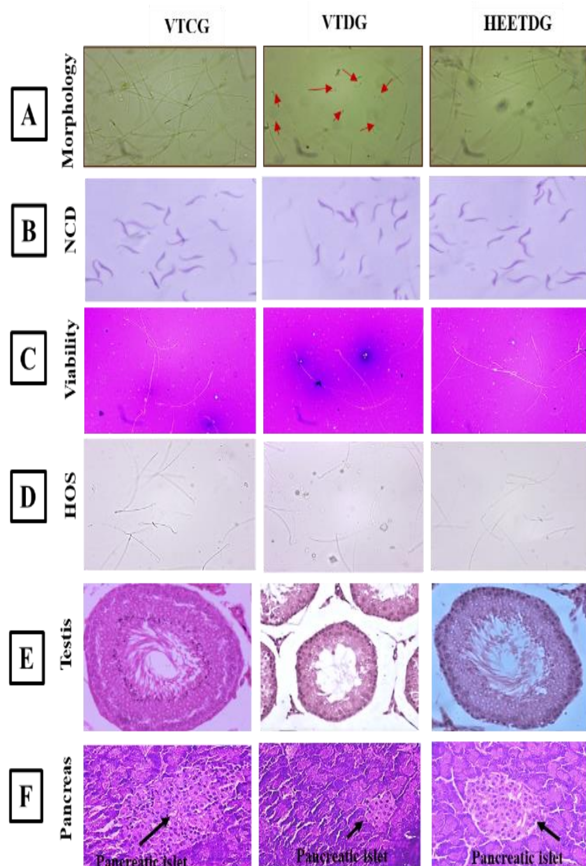

Figure 6. In-vivo effect of *Aloe vera (L.)* in spermiological sensors and histo-architechture of testis and pancreas. [A] Morphology of sperm, [B] Nuclear chromatin decondensation of sperm cells, [C] Viability of sperm, [D] Hypo-osmotic swelling of sperm, [E] Histology of testis by hematoxylin and eosin staining, 400 X [F] Histology of pancreas by hematoxylin and eosin staining, 400 X.

The antioxidant properties of *Aloe vera (L.)* may mitigate oxidative stress by enhancing antioxidant enzyme activities and reducing TBARS levels, key markers of lipid peroxidation, confirmed by the obtained results **(Figure 7).**

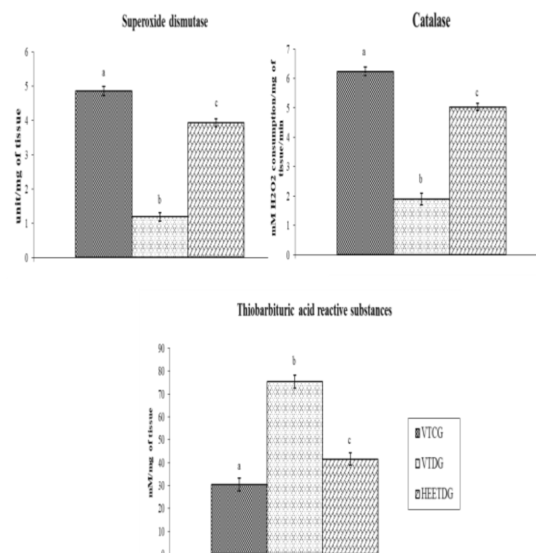

Figure 7. Ameliorative effect *Aloe vera (L.)* on kinetics of anti-oxidant enzyme activities and TBARS level. Statistical tool 'Analysis of Variance' followed by Multiple Comparison Student's Two tail t-test was used. Each column represents Mean ± SEM, sample size (n) = 6. Column with different superscripts (a-c) differ from one another significantly, p<0.05**.**

This normalizes glycation rate and rectifies testicular androgenesis, as evidenced by improved serum testosterone level.

Spermiological analysis of the cauda epididymis gives reflection of the entire result where all the adopted sensors were significantly corrected in the HTG compared to the UDG **(Table 3 and Figure 6).**

Table 3 In-vivo effect of *Aloe vera (L.)* on spermiological sensors in rat

Parameters	VCTG	VCDG	HEETDG
Abnormal sperm head (%)	05.21 ± 0.24^{a}	33.30 ± 0.54^{b}	07.21 ± 0.24^{c}
Motile sperm (%)	75.11± 2.06^{a}	38.13±2.57^{b}	68.15± 2.22^{c}
Viable sperm (%)	83.50 ± 2.68^{a}	43.25 ± 3.65^{b}	70.64 ± 2.69^{c}
HOS (%)	64.52 ± 0.72^{a}	27.98 ± 0.68^{b}	55.41 ± 0.56^{c}
AIS (%)	70.63 ± 0.60^{a}	32.57 ± 0.81^{b}	62.36 ± 0.85^{c}
NCD (%)	73.34 ± 0.69^{a}	57.36 ± 0.74^{b}	65.36 ± 3.25^{c}

Data represents Mean ± SEM, n=6. Values with different superscripts (a-c) in each row differ from one another significantly, p<0.05. ANOVA and then Multiple Comparison Student's Two-tail t-test were conducted.

Histological examination of testicular tissues showed disruption in seminiferous tubules in UDG. Significant improvement was noted in HTG after 28 days of oral gavage treatment (**Figure 6).** The therapeutic effects of *Aloe vera (L.)* extract can be attributed to its multifaceted action on various biological pathways. The extract's antioxidant properties most likely have a major impact on scavenging free radicals,

reducing oxidative stress, thereby protecting sperm DNA and improving sperm quality. *Aloe vera (L.)* contains several bioactive compounds, as shown in **Table 4,** identified by LC-MS study which exhibited potent antioxidant activity, supporting the obtained results.

Table 4 Identification of phytomolecules present in *Aloe vera (L.)* using LC-MS

Retention time (min)	Observed m/z	Ionization mode	Calculated m/z	Formula	Tentative compound	Nature
14.43	316.05	[M−H]-	316.27	C16H12O7	Rhamnetin	Flavonoid
10.47	164.66	[M−H]-	164.16	C9H8O3	Coumaric acid	Phenolic acid
5.68	180.71	[M-H]−	180.16	C9H8O4	Caffeic acid	Phenolic acid
1.75	116.47	[M−H]-	116.07	C4H4O4	Fumaric acid	Organic acid
0.816	150.43	[M + H]+	150.22	C10H14O	Thymol	Phenolic terpenes
25.30	269.63	[M + H]+	270.24	C15H10O5	Apigenin	Flavonoid

Additionally, the anti-inflammatory capabilities of *Aloe vera (L.)* may also contribute to its protective effects by reducing inflammation in the testicular microenvironment, promoting healthier sperm production and function.[29] The improvement in serum insulin and C-peptide levels suggests that *Aloe vera (L.)* extract enhances pancreatic β-cell function, thereby improving overall glucose homeostasis which was confirmed by improvement in the histoarchitehture of pancreas **(Figure 6).** This improvement in glucose metabolism likely alleviates the metabolic stress on the reproductive system, contributing to better spermatogenic outcomes. The results highlighted the potentiality of *Aloe vera (L.)* HEE as a therapeutic agent for alleviating diabetes-induced male infertility. The extract demonstrated significant improvements in sperm motility, viability, DNA integrity, and endocrine function in both ex-vivo and in vivo models. These effects are likely mediated through the modulation of inflammation, oxidative stress, and endocrine-function by *Aloe vera (L.).*

4. Conclusions

Further research is warranted to explore the molecular mechanisms in greater detail and to assess the clinical applicability of these findings in human subjects. The integration of traditional medicinal knowledge with modern scientific approaches offers a promising avenue for developing effective and affordable treatments for diabetes-induced infertility and other related complications.

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6. Notes and References

1. Sun, X.; Yon, D.K.; Nguyen, T.T.; Tanisawa, K.; Son, K.; Zhang, L.; Shu, J.; Peng, W.; Yang, Y.; Branca, F.; Wahlqvist, M. L.; Dietary and other lifestyle factors and their influence on non-communicable diseases in the Western Pacific region. *The Lancet Regional Health – Western Pacific* **2023**, *43*, 1-23.
2. American Diabetes Association.; Diagnosis and classification of diabetes mellitus. *Diabetes Care* **2009,** S62-S67.
3. Pradeepa, R.; Mohan, V.; Epidemiology of type 2 diabetes in India. *Indian Journal of Ophthalmology* **2021**, *69,* 2932-2938.
4. He, Z.; Yin, G.; Li, QQ.; Zeng. Q.; Duan, J.; Diabetes mellitus causes male reproductive dysfunction: A review of the evidence and mechanisms. *In Vivo* **2021,** *35*(5), 2503-2511.
5. Mostafa, T.; Abdel-Hamid, IA.; Ejaculatory dysfunction in men with diabetes mellitus. *World Journal of Diabetes* **2021** *12* (7), 954–974.
6. Yuan, H.; Ma, Q.; Ye, L.; Piao, G.; The traditional medicine and modern medicine from natural products. *Molecules* **2016** *21,* 559-577.
7. Oyebode, O.; Kandala, N. B.; Chilton, P.J.; Lilford, R. J.; Use of traditional medicine in middle-income countries: A WHO-SAGE study. *Health Policy Plan* **2016,** *31*, 984-991.
8. Patil, A.; Nirmal, S.; Pattan, S.; Tambe, V.; Tare, M.; Antidiabetic effect of polyherbal combinations in STZ induced diabetes involve inhibition of α-amylase and α-glucosidase with amelioration of lipid profile. *Phytopharmacology* **2012,** *2*, 46-57.
9. Surjushe, A., Vasani, R.; Saple, D.G.; *Aloe vera*: A short review. *Indian Journal of Dermatology* **2008** *53,* 163-166.
10. Pal, D.; Lohar, P.; Mondal. T.; Ghosh, D.; Amelioration of diabetes associated hypo-testicular co-morbidities in rat by *Aloe vera (L.):* Potent extract selection study. *International Journal of Pharmaceutical Sciences and Drug Research* **2024,** *16(*3), 458-464.
11. Pal, D.; Mitra, D.; Hazra, S.; Ghosh, D.; Protective effect of *Aloe vera (L.)* on diabetes-induced oxidative stress linked spermiological co-morbidity in human and rat: An in-vitro analysis. Reproductive Sciences 2024, In press.
12. Hamza, R. Z.; Al-Baqami, N. M.; Khojah, E.; Mansour, A. M.; E. Al-Motaani, S.; A. Al-Salmi, F.; El-Megharbel, S. M.; Possible antioxidant and antidiabetic effects of *Combretum Molle* extract in a diabetes mellitus experimental model in male rats. *Natural Product Communications* **2021**, *16* (10), 1-10.
13. Bera, T. K.; Chatterjee, K.; Ghosh, D.; Remedial hypoglycemic activity of n-hexane fraction of hydro-methanol extract of seed of *Swietenia mahagoni (L.)* jacq. in streptozotocin-induced diabetic rat: A comparative evaluation. *Journal of Herbs, Spices & Medicinal Plants* **2014**, 21(1), 38–58.
14. Oosterhuis, G. J.; Mulder, A. B.; Kalsbeek-Batenburg, E.; Lambalk, C. B.; Schoemaker, J.; Vermes, I.; Measuring apoptosis in human spermatozoa: A biological assay for semen quality?. *Fertility and Sterility* **2000**, *74* (2), 245-250.

15. Martínez-Luna, G.; Castillo-Cadena, J.; Serment-Guerrero, J. H.; Modified procedure to assess DNA breakage in spermatozoa by means of the comet assay. Revista Internacional de Contaminacion Ambiental **2015**, *31* (1), 39-45.
16. Sarkar, R.; Ghosh, P.; Tripathy, A.; Ghosh, D.; Correction of diabetes-induced testicular dysfunction by a hydro-methanol (60:40) extract of *Curcuma amada* rhizomes: A dose-dependent study. *Journal of Food Biochemistry* **2019,** *43,* 1-13.
17. Gauri, S. S.; Mandal, S. M.; Atta, S.; Dey, S.; Pati, B. R.; Novel route of tannic acid biotransformation and their effect on major biopolymer synthesis in Azotobacter sp. SSB81. *Journal of Applied Microbiology* **2013**, *114* (1), 84–95.
18. Sokal, R. R.; Rohle, F. J.; Biometry. WH Freeman and Company, **1997**.
19. Hiyoshi, T.; Fujiwara, M.; Yao, Z.; Postprandial hyperglycemia and postprandial hypertriglyceridemia in type 2 diabetes. *Journal of Biomedical Research* **2017**, *33* (1), 1–16.
20. Tian, Y.; Song, W.; Xu, D.; Chen, X.; Li, X,; Zhao, Y.; Autophagy induced by ROS aggravates testis oxidative damage in diabetes via breaking the feedforward loop linking p62 and Nrf2. *Oxidative Medicine and Cellular Longevity* **2020**, *2020*, 1-9.
21. Ghafarizadeh, A. A.; Malmir. M.; Naderi, N. S.; Faraji, T.; Ebrahimi, Z.; The effect of vitamin E on sperm motility and viability in asthenoteratozoospermic men: In vitro study. *Andrologia* **2020**, *53* (1), 1-6.
22. Tourmente, M.; Villar-Moya, P.; Rial, E.; Roldan, E. R.; Differences in ATP generation via glycolysis and oxidative phosphorylation and relationships with sperm motility in mouse species. *Journal of Biological Chemistry* **2015,** *290* (33), 20613-20626.
23. Niki, E.; Yoshida, Y.; Saito, Y.; Noguchi, N.; Lipid peroxidation: Mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications* **2005,** *338* (1), 668–676.
24. Sadik, C. D.; Sies, H.; Schewe, T.; Inhibition of 15-lipoxygenases by flavonoids: Structure–activity relations and mode of action. *Biochemical Pharmacology* **2003**, *65*(5), 773–781.
25. Kannan, K.; Jain, S. K.; Oxidative stress and apoptosis. Pathophysiology **2000**, *7* (3), 153-163.
26. Bittner-Schwerda, L.; Malama, E.; Siuda, M.; van Loon, B.; Bollwein, H.; The sperm chromatin structure assay does not detect alterations in sperm chromatin structure induced by hydrogen peroxide. *Animal Reproduction Science* 2022, **247,** 107094.
27. Günther, M.; Dabare, S.; Fuchs, J.; Gunesch, S.; Hofmann, J.; Decker, M.; Culmsee, C.; Flavonoid–phenolic acid hybrids are potent inhibitors of ferroptosis via attenuation of mitochondrial impairment. *Antioxidants* **2023,** *13* (1), 1-28.
28. Manukumar, H. M.; Shiva, Kumar. J.; Chandrasekhar, B.; Raghava, S.; Umesha, S.; Evidences for diabetes and insulin mimetic activity of medicinal plants: Present status and future prospects. *Critical Reviews in Food Science and Nutrition* **2017,** *57*(12), 2712-2729.
29. Paul, S. U.; Dutta, S. O.; Chaudhuri, T. K.; Bhattacharjee, S. O.; Anti-inflammatory and protective properties of *Aloe vera* leaf crude gel in carrageenan induced acute inflammatory rat models. *International Journal of Pharmacy and Pharmaceutical Sciences* **2014**, *6*(9), 368-371.

7. About the author(s)

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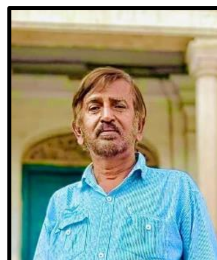

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