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Original Research Article

Attenuation of diabetes linked testicular co-morbidity by *Aloe vera*: A dose-dependent transection study

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Abstract

Objective: The study demonstrated the management efficacy of *Aloe vera* leaf gel extract against diabetes induced testicular co-morbidity in rat.

Materials and methods: Healthy and fertile rats were allowed for diabetes induction by streptozotocin injection at 40 mg dose/kg of somatic weight. Diabetic rats were treated for 28 days at 10, 20, 40 mg doses of hydro-ethanol (40:60, v/v) extract of *Aloe vera* gel or metformin at 2 mg/100 g of somatic weight as positive-control. On 29th day, rats were euthanized and sacrificed. The ameliorative effects were assessed covering glycemic, androgenic, spermatogenic, oxidative stress, histological, genomic and toxicity parameters following established methods. This recovery was compared against diabetic and positive-control groups.

Results: Glucose-6-phosphate dehydrogenase, antioxidant and key androgenic enzyme activities, serum testosterone level and values of sperm parameters were diminished, confirming testicular co-morbidity in diabetes. Simultaneously, fasting blood sugar level, glucose-6-phosphatase activity, seminal vesicular fructose, testicular cholesterol, lipid peroxidation metabolite's level and toxicity sensors were elevated in diabetic group. Significant amelioration ($p<0.05$) in these parameters along with improved gene expression of testicular Δ^5,3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, Bax, and Bcl2 were observed in extract or metformin treated positive-control group, supported by histoarchitectures of testis, pancreas, and liver. The recovery percentages of the sperm parameters were 15-30% more in 20 mg, the maximum effective dose treated group than the positive-control group. The phytocompounds were characterized by GC-MS study.

Conclusion: *Aloe vera* extract may be used as an alternative to the gold standard anti-diabetic drug from the view point of cafeteria choice.

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Introduction

Diabetes mellitus (DM) has a profound negative impact on quality of life where testicular co-morbidity is notifiable one (Maresch et al. 2019). The prevalence of low fertility rate increases in the community with the elevation in male infertility, where one of the factors may be the occurrence of diabetes, more common in men than women (Kautzky-Willer et al. 2023). These detrimental alterations in diabetes are developed through subsequent noxious events in pre-testicular, testicular, and post-testicular levels which are inadequately comprehended due to a lack of in-depth studies (Condorelli et al. 2018). Spermatocytogenesis involves a series of mitotic and meiotic divisions and then metamorphosis known as spermiogenesis, collectively referred as spermatogenesis. Rat's spermatogenesis covered 14 stages which is completed in four cycles. Seventh stage in the cycle is considered for spermatokinetic study which is consist of A-type of Spermatogonia (ASg), pre-Leptotene Primary Spermatocytes (pLPSc), mid-Pachytene Primary Spermatocytes (mPPSc) and step 7 Spermatids (s7Sd). By elongation and maturation, s7Sd transform into functional spermatozoa. Each cycle of the seminiferous epithelium lasts roughly for 12 to 14 days, and for the study on the valid and accurate quantitative changes in the count of concerned germ cell generations at stage VII, duration of experiment for at least two rounds of the cycle should be allowed for obtaining the correct results in this concern (Griswold 2016; Sarkar et al. 2019).

The consequence of testicular dysfunctions in DM has less or no remedy through available drugs as few studies proclaimed that the first-line drugs of diabetes treatment adversely interfere the testicular function and develop subfertility (Alzain et al. 2021), though the statement is controversial **(**Annie et al. 2020). Synthetic drugs have remarkable side effects on different organs apart from the target ones. Therefore, with the steady elevation in DM cases, there is an urgent need for an alternative safe and conflictless therapeutic agent that can rectify testicular co-morbidities along with the remediation of DM. From that perspective, herbal medicine has become an emerging remedy and is mushrooming worldwide due to its less toxic properties (Nasar 2017). Pharmacologists are also endeavoring to develop a therapeutic agent with multifarious targets for correcting multifactorial disorders by eliminating the ongoing trend of the 'one disease- one target – one drug' paradigm and trying to establish the treatment strategy covering 'one drug multi-organ therapy' **(**Lillich et al. 2021).

Aloe vera under *Asphodelaceae* (Liliaceae) family, has notably conquered reputation in Chinese medicine (Gong and Lu 2015) as well as Ayurveda and Homeopathy in Indian traditional medicine (Sahu et al. 2013). The derived gel from *Aloe vera* is widely used to treat wounds, burns, skin disorders, digestive problems, chronic constipation, and poor appetite **(**Olugbenga et al. 2011). Though few studies highlighting its negative influence on male fertility (Bakeer et al. 2021), while most of the observations reflecting its potent protective action against testicular injuries caused by pharmacological and toxic insults (Behmanesh et al. 2018; Erfani Majd et al. 2021). Such conflicting outcomes demand in-depth exploration into its mechanistic pathways and contextual applicability. A pilot investigation was performed to determine the effective solvent extract of *Aloe vera* leaf gel that can rectify diabetes along with diabetes-linked testicular disruptions. Hydro-ethanol (40:60) extract (20 mg/100 g body weight) demonstrated a favorable effect in this concern (Pal et al. 2024). So, in continuation, this experiment was aimed to explore the optimum dose covering the lower as well as higher doses of the pilot work and the mechanism against diabetes-induced testicular disruption in albino rats, may be a step for torching the herbal

pharmaceutical industries for concerned drug development. Moreover, this work also fulfil the strategy of 'one drug-multi complications therapy' which is not till elucidated in this aspect **(**Lillich et al. 2021).

Materials and Methods

Extraction procedure

Fresh, mature *Aloe vera* leaves were gathered in rainy season, validated by Botany Department, Vidyasagar University (Herbarium number- *Aloe vera (L.)*/VU/Bio/06/19), washed in double-distilled water, and the inner gel was drawn. One liter of hydro-ethanol (40:60) solvent was combined with 500 ml of collected gel and processed as per published method (Pal et al. 2024).

Design of the experiment

A schematic presentation of experimental protocol is shown in Figure 1. The Institutional Animal Ethics Committee (IAEC) approved the experiment (VU/IAEC-I/DG-1/3-15/19). Rats were acclimatized in ambient temperature (25± 2^{o}C), regulated moisture, and a light/dark cycle at 1:1 of 24 hours duration for 10 days in animal keeping center of our institute. Standard rat chow and unrestricted access to water were provided. Experimental rats, two months of age, 120 ± 10 g in somatic weight, were selected. After overnight fasting, a single dose of 40 mg streptozotocin (STZ) (Sisco Research Laboratories Pvt. Ltd., Mumbai, India) injection into the leg muscle was given, dissolved in 1000 µl of citrate buffer having pH 4.5/ kg somatic weight (Sarkar et al. 2019; Pal et al. 2024). At that time, same volume of only citrate buffer (pH 4.5) was injected to age and weight-matched control group's rats. In a gap of 72 hours, fasting blood sugar (FBS) levels of rats were measured. The levels within 250 -350 mg/dl were granted for selection of experimental diabetic animals, whereas rats of control group having FBS levels of 75±5 mg/dl were considered as normoglycemic for this experiment.

Rats were allotted randomly into five experimental groups, six rats in each. Day seven after STZ injection was scheduled as the initiation of extract or metformin treatment as positive control and continued for next 28 days. Before and after 2 hours of extract or metformin treatment, food supply was strictly restricted to prevent phytomolecule-nutrient or drug-nutrient interaction, if any. The treatment regimen was as follows.

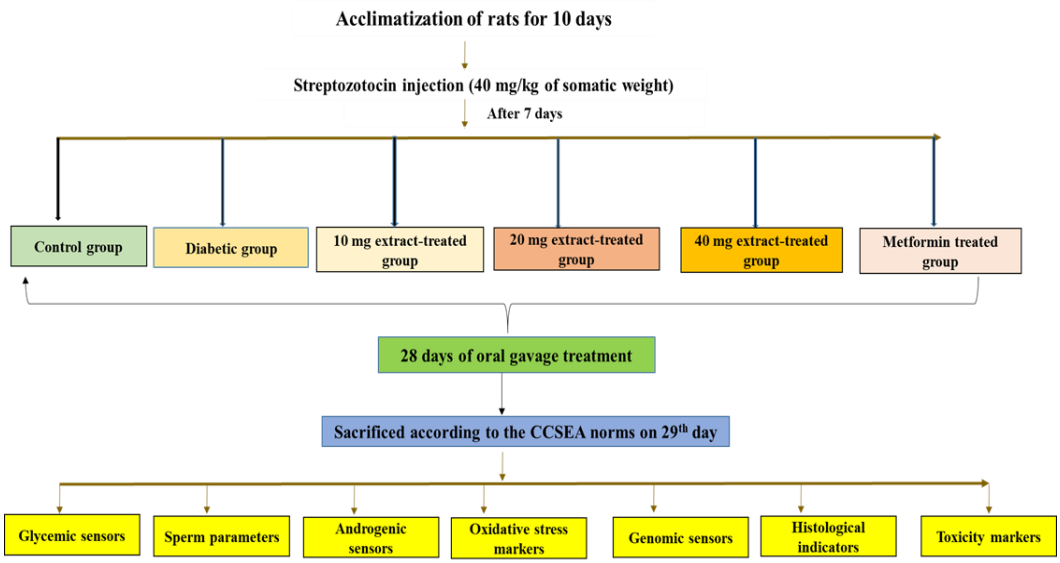

Figure 1. Schematic diagram of experimental protocol

Control group

The rats received a daily oral dose of half ml of deionized water/100 g of somatic weight for 28 days. The treatment was done daily at 9 AM, with food restricted for two hours before and after the treatment.

Diabetic group

For induction of DM of the rats under this group, each rat was subjected to single dose of intramuscular injection of STZ (40 mg/kg of somatic weight in 1 ml citrate buffer), 7 days prior to the experiment. During experimentation diabetic rats were provided with half ml of deionized water orally/100 g of somatic weight/day for 28 days along with food supply restriction as previous group.

Diabetic + 10 mg extract-treated group

Rats in this group were administered hydro-ethanol extract of *Aloe vera* at 10 mg/half ml of deionized water/100 g of somatic weight/day through oral route by gavage for next 28 days. Extract treatment was conducted at 9 AM per day without providing food before and after 2 hours of treatment.

Diabetic + 20 mg extract-treated group

Oral gavage feeding was done with 20 mg hydro-ethanol extract of *Aloe vera* solubilized in half ml deionized water/100 g of somatic weight/day for next 28 days. The extract was given each day at 9 AM, with food deprivation enforced for 2 hours preceding and succeeding the treatment.

Diabetic + 40 mg extract-treated group

The potent extract of *Aloe vera* at 40 mg was solubilized in half ml deionized water and administered orally/100 g of somatic weight/ day for next 28 days, accompanied by restriction of food supply, similar to the preceding group.

Diabetic + Metformin treated group (positive control)

Individual rat of this group was administered orally with 2 mg of metformin dissolved in half ml of deionized water/100 g of somatic weight/day for next 28 days as per literature (Adeneye et al. 2011). Metformin was given at 9 AM. Food was withheld for 2 hours pre and post treatment of metformin.

Proper guideline of Committee for Control and Supervision of Experiments on Animals (CCSEA) was followed to sacrifice the rats on 29^{th} day. Serum was separated from collected blood and stored at -20^{0}C. Pancreas, slices of liver, seminal vesicle, epididymis, and testis were dissected out and used for instant histological and biochemical studies as the case may be.

Glycemic sensors

Sugar level in blood after overnight fasting was measured from the caudal vein by a glucometer at an interval of 7 days throughout the experiment. Fifty mg of hepatic tissue was homogenized in 1000 µl of icy 0.1 M phosphate buffered saline (pH 7.4) to adjudicate glucose-6-phosphatase (G-6-P) and glucose-6-phosphate dehydrogenase (G-6-PD) kinetics using published protocols (Bera et al. 2015; Amini et al. 2021).

Sperm parameters

Twenty µl of epididymal washed sperm suspension was used for percentage counting of alive sperms and number of sperm in million/ml under a microscope. Acrosomal intactness status (AIS) was assessed using glass slides pre-coated with gelatin. Spermatozoa formed halos were expressed in percentage count. Assessment of hypo-osmotic swelled (HOS) sperm was performed by exposing these sperm cells to hypo-osmotic fluid, and positive cells were expressed in percentage (Murugesan et al. 2022; Das et al. 2023).

Key androgenic enzymes

Key androgenic enzymes i.e. Δ^5, 3β-HSD and 17β-HSD kinetics were estimated

spectrophotometrically, noting the optical density at 340 nm at half minute of interval for three minutes (Das et al. 2023).

Testicular cholesterol, seminal vesicular fructose (SVF) and testosterone concentration in serum

Cholesterol level in testis and SVF concentration were measured biochemically with a standard procedure. The testosterone level in serum was assessed by enzyme linked immunosorbent assay using kit of Lilac Medicare (P) Ltd, Mumbai, India (Sarkar et al. 2019).

Oxidative stress and toxicity markers

Superoxide dismutase (SOD), peroxidase activities and the concentration of substances reactive to thiobarbituric acid (TBARS) in hepatic tissue, testis, epididymis, as well as sperm pellet were measured (Bera et al. 2015; Arab et al. 2023).

Turnover rates by serum transaminases for glutamate-oxaloacetate (SGOT) and glutamate-pyruvate (SGPT) were assessed spectrophotometrically. Serum creatinine and urea levels were measured in a semi-auto analyzer (Bera et al. 2015; Bakour et al. 2021).

Genomic expression study

"High Pure Tissue Kit" (Roche, Mannheim, Germany) was used for the extraction of mRNA from the concerned tissues. Male gonadal key hydroxy dehydrogenases, Bax, and Bcl-2 gene expressions were studied by Light Cycler 480 II (Roche Diagnostic, Germany) using cyber green (Sarkar et al. 2019).

Histological study

Paraffin-embedded blocks of testis, pancreas, and liver were sectioned at a thickness of 5 µm using a semi-automatic microtome and stained by hematoxylin-eosin. Adjudication about diameter of seminiferous tubule (STD) was performed using a software named as Dewinter Caliper Pro 3.0, and sperm cells of different generations at stage VII of spermatogenic cycle were quantified using Abercrombie's formula (Sarkar et al. 2019). Identification of ASg was done by their large size, round shape and location near the basement membrane of the wall of seminiferous tubule with dark, less condensed nuclei, while pLPSc were slightly smaller with more condensed chromatin and were positioned just above the spermatogonia. Irregularly shaped, darkly stained nuclei, indicative of chromosomal synapsis were the features of mPPSc whereas s7Sd were the smallest cells with densely packed, dark nuclei and minimal cytoplasm, located near the lumen (Sarkar et al. 2019; Achi et al. 2024).

Phytomolecule analysis utilizing Gas Chromatography-Mass Spectrometry (GC-MS)

A Perkin Elmer Clarus 600C MS integrated with a 30 m × 0.25 mm i.d. fused silica capillary column (0.25 µm film thickness) was utilized. Compounds in the extract were identified through split injection (1:120 ratio) and compared to standards available in existing internet-based phytomolecule library (Mainlab and Replib) (Shettar et al. 2017).

Statistical analysis

Statistical analysis was done using mean ± SEM at significance level $p<0.05$ through "Analysis of Variance" (ANOVA) Model-I, one way, followed by "Multiple comparison Student's one-tail 't-test" to know the significance of the observed co-relation (Das and Das 2005).

Results

Glycemic sensors

The FBS level and hepatic G-6-P activity were elevated, while G-6-PD activity was reduced in diabetic group against the control group. A notable ($p<0.05$) improvement was noticed with all the doses of the extract, with maximum effect at 20 mg dose though metformin

demonstrated superior improvement than extract-treated groups (Figure 2 and 3).

HOS-positive, and intact acrosome sperm, along with lower sperm count against the control group. Treatment especially at 20 mg dose, significantly ($p<0.05$) corrected these parameters, exceeding metformin's effects (Table 1).

Sperm parameters

The diabetic group showed significantly ($p<0.05$) reduced viable,

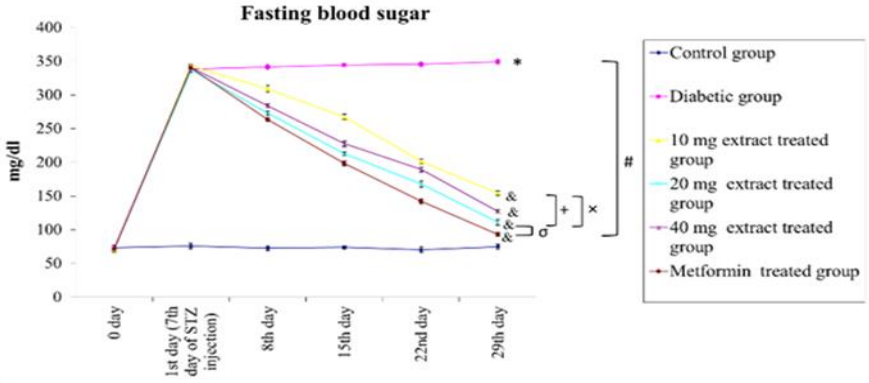

Figure 2. Corrective effect of *Aloe vera* extract on FBS level. Each point of the line diagram expressed mean ± SEM (n = 6). ANOVA followed by "Multiple comparison student's one-tail t-test". Control vs diabetic: '*' represents ($p<0.05$); control vs treated groups: '&' represents ($p<0.05$); diabetic vs treated groups: '#' represents ($p<0.05$); 20 mg vs 10 mg extract treated groups: '+' represents ($p<0.05$); 40 mg vs 10 and 20 mg extract treated groups: '×' represents ($p<0.05$); 20 mg vs metformin treated: 'σ' represents ($p<0.05$).

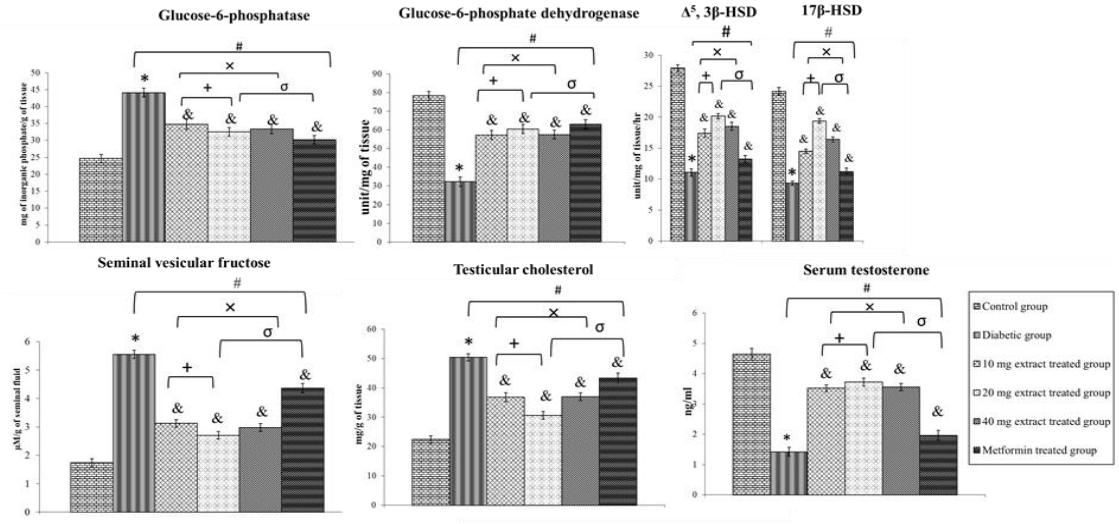

Figure 3. Correction in carbohydrate metabolic and androgenic key enzymes activities, seminal vesicular fructose, testicular cholesterol and serum testosterone levels after *Aloe vera* extract or metformin treatment. Data represented as mean ± SEM, n = 6. ANOVA followed by "Multiple comparison Student's one-tail t-test". Control vs diabetic: '*' represents ($p<0.05$); control vs treated groups: '&' represents ($p<0.05$); diabetic vs treated groups: '#' represents ($p<0.05$); 20 mg vs 10 mg extract treated groups: '+' represents ($p<0.05$); 40 mg vs 10 and 20 mg extract treated groups: '×' represents ($p<0.05$); 20 mg vs metformin treated: 'σ' represents ($p<0.05$).

Table 1. Dose-dependent remedial effect of *Aloe vera* hydro-ethanol extract on sperm parameters and spermatokinetics

Groups	**Sperm count (million/ml)**	**Sperm viability (%)**		**% of Sperms with hypo-osmotic swelling (HOS)**	**% of Sperms with acrosome intactness**	**Different generations of germ cells at stage VII**			
		Alive	**Dead**			**ASg**	**pLPSc**	**mPPSc**	**s7Sd**
Control group	31.50±0.61	86.33 ± 1.75	13.12 ± 0.92	63.16 ± 0.79	72.66 ± 0.76	0.65 ± 0.05	21.55 ± 0.23	24.77 ± 0.51	78.21 ± 2.13
Diabetic group	10.17±0.57*	46.16 ± 1.68*	53.84 ± 1.23*	29.33 ± 0.49*	30.16 ± 0.79*	0.31 ± 0.07*	8.11 ± 0.41*	7.23 ± 0.16*	15.58 ± 1.19*
10 mg extract treated group	18.83±0.30$^{*,\&,\#,+,\times}$	68.16 ±1.32*$^{,\&,\#,+,\times}$	31.84 ± 1.36$^{*,\&,\#,+,\times}$	48.33 ± 0.76$^{*,\&,\#,+,\times}$	56.5 ± 1.05$^{*,\&,\#,+,\times}$	0.54 ± 0.04$^{*,\&,\#,+,\times}$	16.42 ± 0.27$^{*,\&,\#,+,\times}$	18.53 ± 0.43$^{*,\&,\#,+,\times}$	50.04 ± 1.41$^{*,\&,\#,+,\times}$
20 mg extract treated group	25.5±0.42$^{*,\&,\#,\times}$	71.33 ± 1.23$^{*,\&,\#,\times}$	28.67 ± 1.19$^{*,\&,\#,\times}$	53.66 ± 0.55$^{*,\&,\#,\times}$	61.16 ± 0.47$^{*,\&,\#,\times}$	0.56 ± 0.05$^{*,\&,\#,\times}$	19.47 ± 0.33$^{*,\&,\#,\times}$	22.11 ± 0.23$^{*,\&,\#,\times}$	59.69 ± 2.63$^{*,\&,\#,\times}$
40 mg extract treated group	26.5±0.63$^{*,\&,\#}$	67.16 ± 1.51$^{*,\&,\#}$	32.84 ± 1.27$^{*,\&,\#}$	48.5 ± 0.76$^{*,\&,\#}$	57.66 ± 0.42$^{*,\&,\#}$	0.58 ± 0.14$^{*,\&,\#}$	18.32 ± 0.12$^{*,\&,\#}$	19.01 ± 0.47$^{*,\&,\#}$	51.32 ± 2.12$^{*,\&,\#}$
Metformin treated group	14.12 ± 0.50$^{*,\&,\#,\sigma}$	57.27 ± 1.85$^{*,\&,\#,\sigma}$	42.73 ± 2.12$^{*,\&,\#,\sigma}$	36.21 ± 0.52$^{*,\&,\#,\sigma}$	41.18 ± 0.53$^{*,\&,\#,\sigma}$	0.40± 0.05$^{*,\&,\#,\sigma}$	12.02 ± 0.43$^{*,\&,\#,\sigma}$	11.19 ± 0.41$^{*,\&,\#,\sigma}$	27.97 ± 1.10$^{*,\&,\#,\sigma}$

Data represented as mean ± SEM, n = 6. ANOVA followed by "Multiple comparison Student's one-tail t-test". Control vs diabetic: '*' represents ($p<0.05$); control vs treated groups: '&' represents ($p<0.05$); diabetic vs treated groups: '#' represents ($p<0.05$); 20 mg vs 10 mg extract treated groups: '+' represents ($p<0.05$); 40 mg vs 10 and 20 mg extract treated groups: '×' represents ($p<0.05$); 20 mg vs metformin treated: 'σ' represents ($p<0.05$).

Testicular cholesterol, SVF and serum testosterone level

The diabetic group had elevated SVF and testicular cholesterol but reduced testosterone levels against the control group. Extract treatment, particularly at 20 mg, significantly ($p<0.05$) restored these parameters, surpassing the efficacy of metformin (Figure 3).

Δ^5, 3β-HSD and 17β-HSD

A substantial decline in the kinetics of both the enzymes in the diabetic group which were significantly ($p<0.05$) restored by the extract or metformin treatment, showing maximum efficacy at 20 mg dose, exceeding the corrective effect of metformin (Figure 3).

Oxidative stress and toxicity markers

The diabetic group exhibited reduced SOD and peroxidase activities, with elevated TBARS in liver, testis, epididymis, and sperm pellet than the control group. Elevated levels of toxicity markers (SGOT, SGPT, urea, and creatinine) in diabetic group than the control group were reversed at significant ($p<0.05$) level in the extract or metformin-treated group, with maximum recovery at 20 mg dose (Figure 4 and Figure 5).

Genomic expression study

The diabetic group exhibited up-regulated testicular Bax and down-regulated Bcl-2, Δ^5,3β-HSD and 17β-HSD gene expression against the control group, were significantly ($p<0.05$) rectified by extract or metformin treatment at the transcript level, focusing maximum efficacy at 20 mg dose (Figure 6).

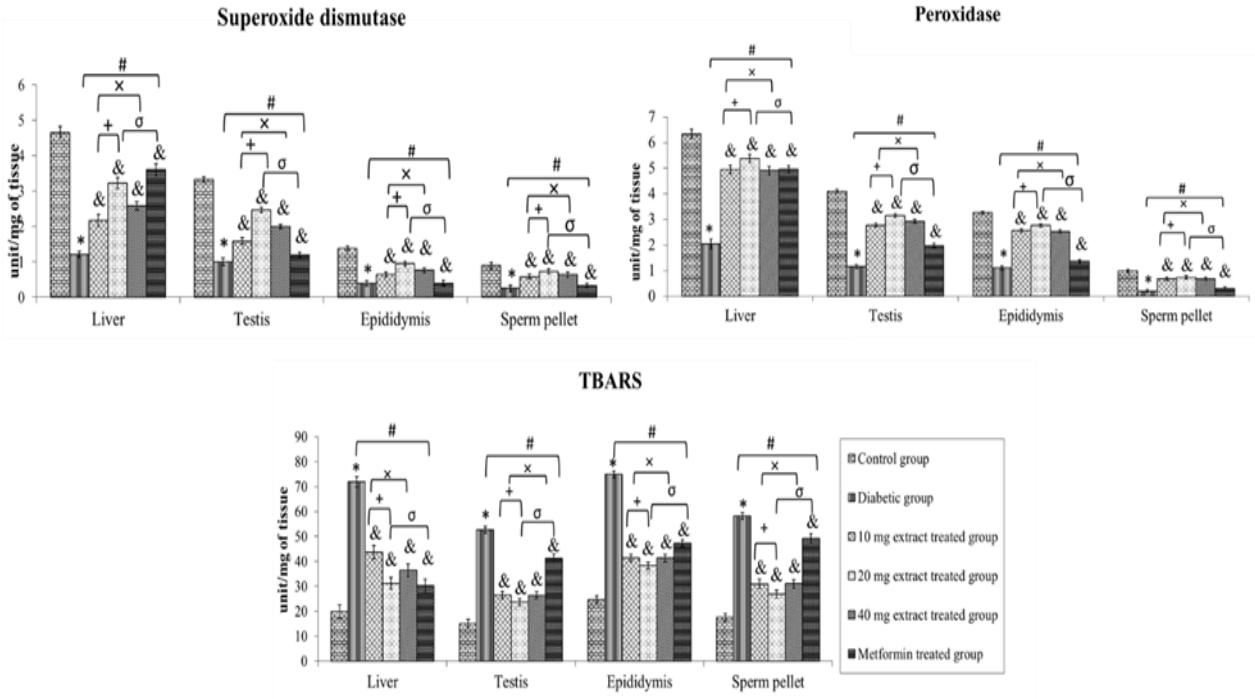

Figure 4. Remedial effect of *Aloe vera* or metformin on oxidative stress markers. Each bar represented mean ± SEM, n = 6. ANOVA followed by "Multiple comparison Student's one-tail t-test". Control vs diabetic: '*' represents ($p<0.05$); control vs treated groups: '&' represents ($p<0.05$); diabetic vs treated groups: '#' represents ($p<0.05$); 20 mg vs 10 mg extract treated groups: '+' represents ($p<0.05$); 40 mg vs 10 and 20 mg extract treated groups: '×' represents ($p<0.05$); 20 mg vs metformin treated: 'σ' represents ($p<0.05$).

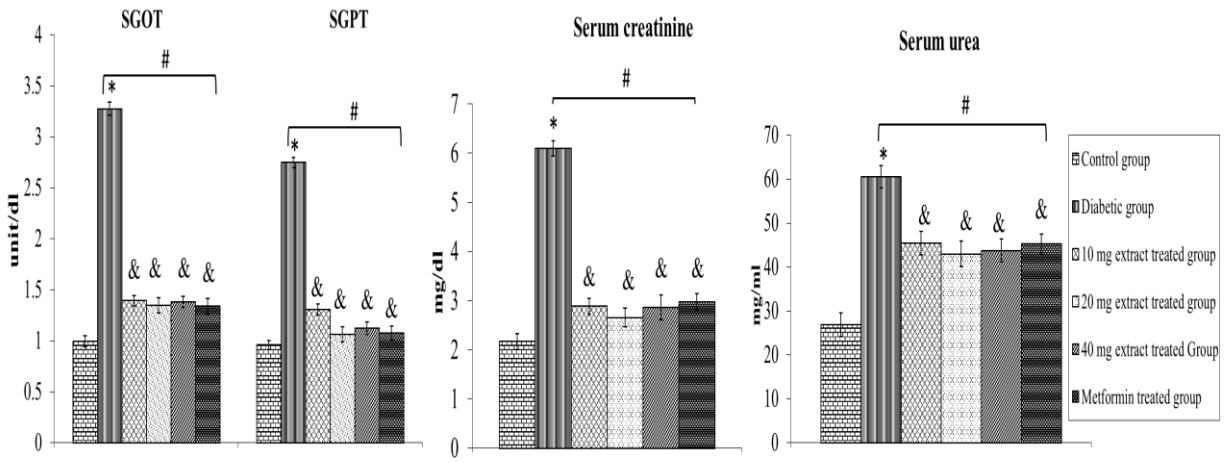

Figure 5. Activities of SGOT, and SGPT, serum creatinine and urea levels in STZ-induced diabetic rats and its amendment by *Aloe vera* extract treatment. Bars expressed as mean ± SEM (n = 6). ANOVA followed by "Multiple comparison Student's one-tail t-test". Control vs diabetic: '*' represents ($p<0.05$); control vs treated groups: '&' represents ($p<0.05$); diabetic vs treated groups: '#' represents ($p<0.05$).

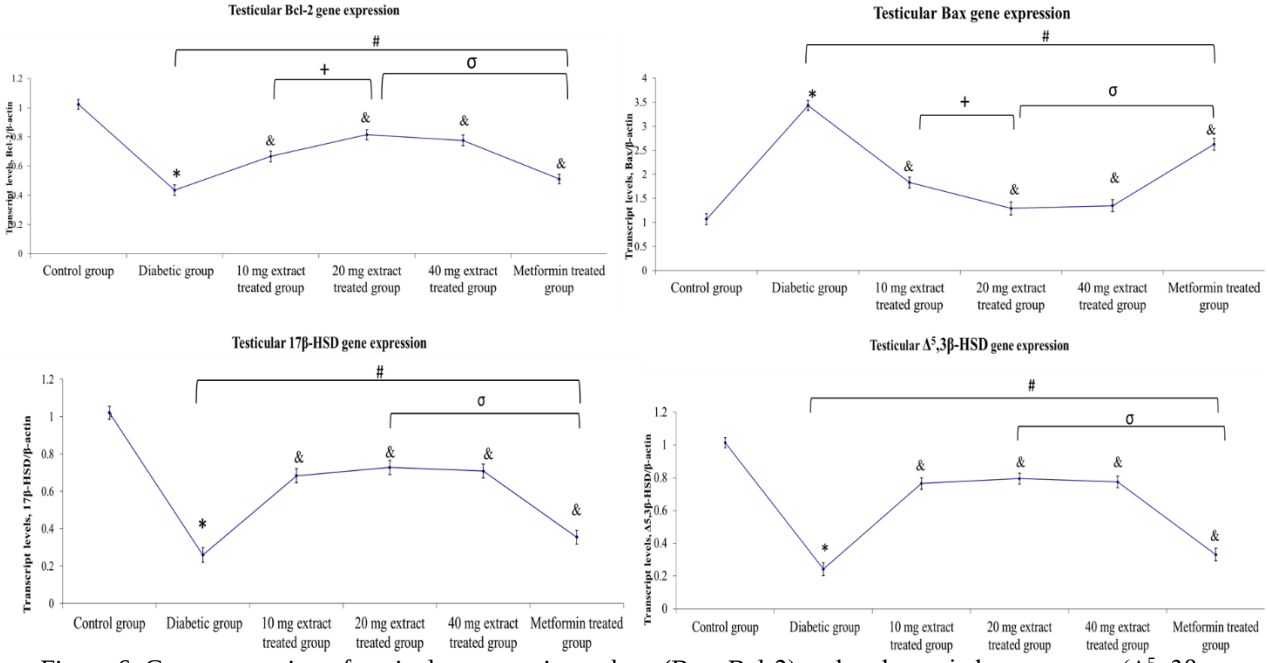

Figure 6. Gene expression of testicular apoptotic markers (Bax, Bcl-2) and androgenic key enzymes (Δ^5, 3β-HSD, and 17β-HSD) by qRT-PCR in STZ induced diabetic group and its rectification after extract or metformin treatment. Each point of the line diagram expressed as mean ± SEM (n = 6). ANOVA followed by "Multiple comparison Student's one-tail t-test". Control vs diabetic: '*' represents ($p<0.05$); control vs treated groups: '&' represents ($p<0.05$); diabetic vs treated groups: '#' represents ($p<0.05$); 20 mg vs 10 mg extract treated groups: '+' represents ($p<0.05$); 20 mg vs metformin treated groups: 'σ' represents ($p<0.05$).

Histological study

Count of ASg, pLPSc, mPPSc, and s7Sd at stage VII of male germ cell cyclic process operated in germinal epithelium of seminiferous tubule (Table 1) along with STD presented a significant deteriorating levels in diabetic group against the control group, corrected significantly by extract treatment and more rectified in comparison to metformin treatment. Pancreatic islet size was decreased along with more fat deposition in the central vein of the hepatic lobule in diabetic group than the control group, were rectified by extract or metformin treatment (Figure 7).

GC-MS analysis

The presence of 30 compounds were listed in Table 2. Out of these, seven compounds (1,5-Pentanediol, 3-methyl-; 7-Heptadecene, 17-chloro-; 7-Heptadecene, 1-chloro-; 4-Undecene, (Z)-; Cyclopentaneethanol,beta.,2,3-trimethyl-; Cholestan-3,26-diol-22-one; Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1s-(1.alpha.,2.beta.,5.alpha.)]-) were present at maximum level having anti-diabetic, anti-oxidative and insulin resistance recovery activity, needed for extensive study for their specific functional activity assessment.

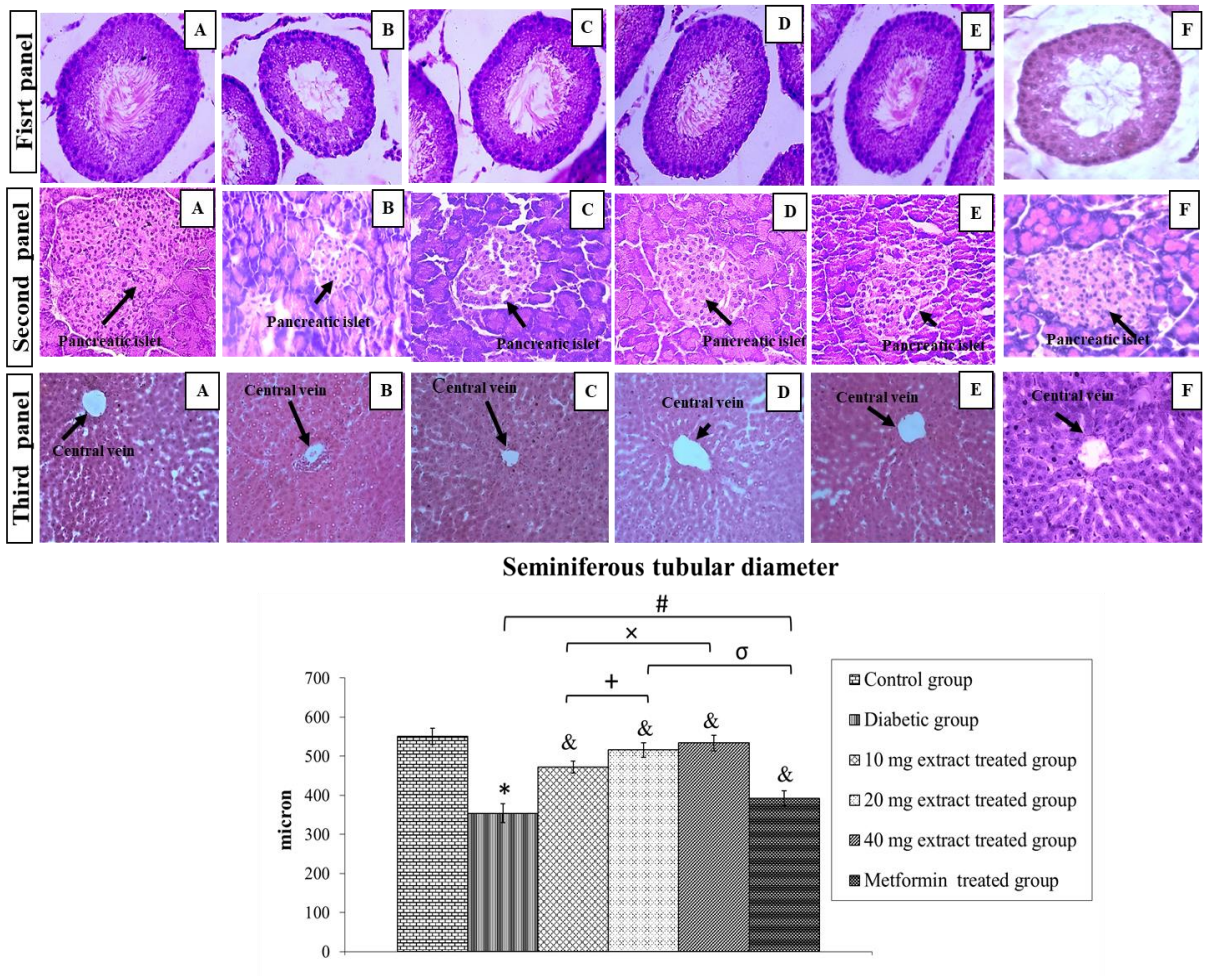

Figure 7. First panel focused the histology of seminiferous tubule with diameter of the tubule and population density of matured spermatozoa in lumen and immature sperm along the side of lumen in control (A) which were semi-quantitively decreased in diabetic group (B). After supplementation of the said extract at three different doses or metformin, the diameter of the tubule and population density of matured spermatozoa along with different generations of immature germ cells all were recovered towards the control with optimum recovery at 20 mg dose (C-F). Second panel focused histomorphological alteration in 'Islets of Langerhans' along with population density of Islet's cells in qualitative aspects in control group (A) which were decreased in diabetic group (B), rectified by treatment of the said extract or metformin (C-F) with optimum correction by 20 mg dose. Third panel enlighted the histoarchitechture of liver focusing central vein and polyhedral hepatocytes arrangement in cord like fashion in lobule in control (A). In diabetic group, the luminal size of hepatic central vein was reduced remarkably indicated by arrow along with dearrangement in hepatocytes cords in lobule and fat deposition (B). All were corrected by extract or metformin treatment (C-F). Quantitative aspects of seminiferous tubular diameter after extract or metformin treatment. Bars were expressed as mean ± SEM (n = 6). Model-I one-way ANOVA followed by "Multiple comparison Student's one-tail t-test". Control vs diabetic: '*' represents ($p<0.05$); control vs treated groups: '&' represents ($p<0.05$); diabetic vs treated groups: '#' represents ($p<0.05$); 20 mg vs 10 mg extract treated groups: '+' represents ($p<0.05$); 40 mg vs 10 and 20 mg extract treated groups: '×' represents ($p<0.05$); 20 mg vs metformin treated: 'σ' represents ($p<0.05$).

Table 2. Phyto-compounds present in the hydro-ethanol (40:60) extract of *Aloe vera* by GC-MS

Peak No	Retention time (min)	Compound name	Molecular weight	Formula	Area %
1	4.01	1,5-Pentanediol, 3-methyl-	118	C6H14O2	1.45
2	4.08	4-Tridecene, (z)-	182	C13H26	0.73
3	4.13	4-Tetradecene, (e)-	196	C14H28	0.57
4	4.19	2-Decen-1-ol, (e)-	156	C10H20O	0.35
5	4.22	Erucic acid	338	C22H42O2	0.73
6	4.28	4-Tetradecene, (z)-	196	C14H28	0.35
7	4.33	8-Heptadecene, 1-chloro-	272	C17H33Cl	0.34
8	4.39	8-Oxabicyclo[5.1.0]octane	112	C7H12O	0.36
9	4.82	Cis-2-methyl-7-octadecene	266	C19H38	0.36
10	5.54	1,2:4,5:9,10-Triepoxydecane	184	C10H16O3	0.73
11	5.60	3-Methyl-2-(2-oxopropyl)furan	138	C8H10O2	0.50
12	6.30	7-Heptadecene, 17-chloro-	272	C17H33Cl	3.05
13	6.40	9-Octadecenoic acid, 2,2,2-trifluoroethyl ester	364	C20H35O2F3	0.89
14	10.05	Undecanal	170	C11H22O	0.40
15	14.44	7-Tetradecene	196	C14H28	0.44
16	15.47	2,6,6-Trimethyl-bicyclo[3.1.1]hept-3-ylamine	153	C10H19N	0.84
17	16.05	7-Heptadecene, 1-chloro-	272	C17H33Cl	3.22
18	16.70	Dodecanal	184	C12H24O	0.63
19	17.16	4-Undecene, (z)-	154	C11H22	3.54
20	17.43	Cyclopentaneethanol, beta.,2,3-trimethyl-	156	C10H20O	1.19
21	17.72	Cyclopentane, 2-(1-hydroxy-2-propyl)-1,3-dimethyl-	156	C10H20O	0.45
22	18.86	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	262	C19H34	0.49
23	19.30	Cyclohexaneethanol, beta.,4-dimethyl-, cis-	156	C10H20O	0.29
24	19.96	Cholestan-3,26-Diol-22-one	418	C27H46O3	1.36
25	20.71	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1S-(1.alpha.,2.beta.,5.alpha.)]-	138	C10H18	1.72
26	22.99	Cyclohexaneethanol, beta.,4-dimethyl-, trans-	156	C10H20O	0.51
27	25.59	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-	152	C10H16O	0.32
28	25.61	Glutaric acid, (cyclohex-3-enyl) methyl cyclohexylmethyl ester	322	C19H30O4	0.27
29	26.02	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-	152	C10H16O	0.60
30	42.21	Cyclohexanemethanol, 4-(1-methylethyl)-, cis-	156	C10H20O	0.28

Discussion

The extract corrected the elevated FBS, G-6-P hyperactivity, and G-6-PD hypoactivity in the diabetic group due to insulinotropic effects or phytomolecule-gene interactions, supported by other studies (Ansari et al. 2022). It has validated by the recovery of pancreatic islet and hepatic histoarchitecture, as the liver is a central metabolic organ (Rui 2014) and hepatocytes are key stem cells for islet beta-cell generation (Wang et al. 2021).

Diabetes is strongly linked to reactive oxygen species (ROS) generation, and resulting in low sperm motility, viability, and decreased HOS- and AIS-positive sperm due to the oxidation-peroxidation chain reactions of saturated fatty acids, abundant in sperm cells (Alahmar 2019). This is corroborated by decreased antioxidant enzyme activities and high TBARS levels in liver, sperm pellets and reproductive tissues in the diabetic group. The extract rectified these disruptions, likely through free radical scavenging or quenching (Moukette et al. 2015) and

increased antioxidant enzyme activity via phytochemical-gene interaction, like the other plants (Sharmen et al. 2022). It also managed diabetes-induced spermiological disruption and apoptosis by modulating Bax and Bcl2 gene expression.

Interfering action of diabetes on testicular androgenesis and gametogenesis, evidenced by low serum testosterone along with elevated testicular cholesterol levels, reduced Δ^5,3β-HSD and 17β-HSD activities as well as gene expression of the said enzymes, and depletion of spermatogenic cells at hormone-responsive stage VII of spermatogenesis (Smith and Walker 2014). The extract ameliorated these deficits by modulating androgenic enzyme gene expression possibly through phyto-genomic interaction (Ghosh et al. 2014), restoring testosterone, and rectifying SVF, thereby optimizing fructose metabolism in spermatozoa (Sarkar et al. 2019), supporting its therapeutic efficacy. Histological study of testis also focused that diabetes linked histoarchitechture as noted in microphotographs were rectified by the extract. The comparison of the extract with positive control also focused that sperm parameters were rectified more by the extract than the metformin.

Improvement in serum GOT, GPT activities, levels of serum urea and creatinine in extract-treated group compared to diabetic group focused the curative function of *Aloe vera* against diabetes-associated liver toxicity and nephropathy (Bera et al. 2015), though further research is required.

The present study clearly represents *Aloe vera's* therapeutic potentiality against diabetes-induced testicular insult as well as carbohydrate metabolic dysfunction, supporting its use as a multi-target therapeutic agent. A non-linear dose-response was observed, with 20 mg dose showing maximum efficacy, due to optimal receptor occupancy or the spare receptor hypothesis (Salahudeen and Nishtala 2017). The spermiological protection along with the management of diabetes by this extract was supported by the phytomolecules identified by GC-MS study as two of the identified compounds with high area percentage i.e., 8-heptadene reported as anti-diabetic, bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1s-(1.alpha.,2.beta.,5.alpha.)]- reported as anti-oxidative, and anti-inflammatory activities (Apata et al. 2017; Remya et al. 2022). Additional studies are needed to isolate specific phytomolecule(s) that contribute to such rectifications.

The *Aloe vera* hydro-ethanol extract demonstrates diabetes-associated testicular hypoactivity rectifying potentiality by correcting the concerned sperm parameters. Administering the extract for few months prior to 'Assisted Reproductive Technology (ART)' adoption may significantly optimize sperm quality for improving ART-based conception rates in male-factor infertility cases.

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Conflicts of interest

The authors declare no competing interests.

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Ethical Considerations

The IAEC approved the experimental design conducted on rat. Animal handling and treatment protocol for diabetes induction were executed according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Govt. of India.

Code of Ethics

Ethical approval no.: VU/IAEC-I/DG-1/3-15/19.

Authors' Contributions

DP: Conceptualization, Methodology, Writing (Original draft, Review & Editing), Investigation, Validation, Formal analysis, Data curation
SH: Project administration, Formal analysis
PD: Animal handling, Data curation
DG: Supervision, Conceptualization, Validation, Methodology, Writing (Original draft, Review & Editing)
All the authors read and approved the article

Abbreviations

AIS- Acrosomal Intactness Status
ANOVA- Analysis of Variance
ART- Assisted Reproductive Technology
ASg- A-type of Spermatogonia
CCSEA- Committee for Control and Supervision of Experiments on Animals
DM- Diabetes Mellitus
DST- Department of Science & Technology
FBS- Fasting Blood Sugar
G-6-P- Glucose-6-phosphatase
G-6-PD- Glucose-6-phosphate Dehydrogenase
GC-MS- Gas Chromatography Mass Spectrometry
HOS- Hypo-osmotic Swelling
HSD- Hydroxysteroid Dehydrogenase
IAEC- Institutional Animal Ethics Committee
mPPSc- mid-Pachytene Primary Spermatocytes
pLPSc- pre-Leptotene Primary Spermatocytes
ROS- Reactive Oxygen Species
s7Sd- step 7 Spermatids
SEM- Standard Error of Mean
SGOT- Serum Glutamic Oxaloacetic Transaminase
SGPT- Serum Glutamic Pyruvic Transaminase
SOD- Superoxide Dismutase
STD- Seminiferous Tubular Diameter
STZ- Streptozotocin
SVF- Seminal Vesicular Fructose
TBARS- Thiobarbituric Acid Reactive Substances

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