Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Amelioration of Diabetes Associated Hypo-testicular Co-morbidities in Rat by *Aloe vera (L.)*: Potent Extract Selection Study

Dibya Pal, Pampa Lohar, Tanusree Mondal, Debidas Ghosh*

Molecular Medicine, Nutrigenomics and Public Health Research Laboratory, Department of Biomedical Laboratory Science and Management, Vidyasagar University, Midnapore, West Bengal, India.

ARTICLE INFO

Article history:

Received: 19 April, 2024

Revised: 24 April, 2024

Accepted: 26 April, 2024

Published: 30 May, 2024

Keywords:

Diabetes, Streptozotocin, Oxidative stress, Testicular hypo-function, Multi-target approach.

DOI:

10.25004/IJPSDR.2024.160319

ABSTRACT

The experiment was framed to get the most effective extract of *Aloe vera (L.)* leaf gel for the amelioration of hypo-functional activity of testis in diabetic model rats. Only one frequency of streptozotocin was injected through skeletal muscle at 40 mg/kg body weight for diabetes induction. Treatment with aqueous or ethanol or methanol or hydro-ethanol (40:60) or hydro-methanol (40:60) extract of *Aloe vera (L.)* was continued for 28 days. Rats were euthanized and sacrificed on 29th day. Fasting blood glucose level, kinetics of hexokinase, androgenic key enzymes, and markers of cellular oxidative stress were assessed. The concentration of the sperm per milliliter of epididymal washed fluid, sperm motility, serum testosterone, plasma insulin levels, lipid, and metabolic toxicity sensors were also measured. Significant amelioration ($p < 0.05$) of the negatively deviated above-mentioned parameters and the disrupted histomorphology of testicles towards vehicle-treated control were noted after uninterrupted 28 days of treatment to diabetic rats with the mentioned extracts of *Aloe vera (L.)*. The highest percentage of recovery in the adopted sensors was noted in the hydro-ethanol extract-treated diabetes group than others. Hydro-ethanol extract of the said plant part is potent among all other extracts for correcting such hypo-function of testicles in diabetes.

INTRODUCTION

Male reproductive function is compromised by diabetes mellitus, a major global health concern nowadays.[1,2] Hyperglycemia-induced massive generation of mitochondrial oxygen species of high reactivity (OSHR) can bring several difficulties in different organs, including testis.[3] Reports have demonstrated that chronic hyperglycemia results in alteration of androgenesis, damage of testicular tissue, and impairment of spermatogenesis that ultimately induces male subfertility.[4-6] It is still difficult to determine the exact pathophysiology by which oxidative stress causes male infertility in diabetes. However, literature reflects that increased levels of OSHR frequently impair sperm motility by inducing oxidation reactions of lipids present in the spermatozoal membrane and rupturing the structural integrity of lipid architecture along that membrane.[7]

Market-available anti-hyperglycemic drugs are reported as a potential causative factor for cardiac and gastrointestinal problems. Therefore, investigation on alternative cum safe hypoglycemic agents with multi-target therapeutic effect for this purpose is urgently needed.[8] The increasing demand for medicinal plants is attributed to their preventive and healing properties. *Aloe vera (L.)* is a succulent xerophyte, adaptable and versatile enough to survive in hot and dry climates. The succulence trait of the plant is the reason for an abundance of different phytochemicals.[9] Antioxidant, hepato and gastroprotective, anti-inflammatory, anti-fungal, and wound repairing are few clinical features of the said plant.[10] The gel part of the said plant has been used traditionally for its glucose-lowering effect since immemorial.[11] A dose-dependent pilot study has been conducted by us covering 10 to 40 mg doses where

***Corresponding Author:** Prof. Debidas Ghosh

Address: Molecular Medicine, Nutrigenomics and Public Health Research Laboratory, Department of Biomedical Laboratory Science and Management, Vidyasagar University, Midnapore, West Bengal, India.

Email ✉**:** debidasghosh999@gmail.com

Tel.: +91-9232690993

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

20 mg/100 g of bodyweight (BW) of each extract of *Aloe vera (L.)* showed maximum potency on some representative but sensitive sensors for the management of diabetes and its co-morbidities. On that background information, the present investigation has been framed to unfold the management capability of the specific extract at that optimum dose for diabetes and its linked testicular co-morbidities covering enzymatic, cytological, histological and toxicity sensors.

Materials And Methods

Chemicals and Reagents

Streptozotocin, NADP, ATP, and HEPES were brought from Sigma-Aldrich Diagnostic, Maharashtra, India. Kits for lipid and toxicity profile assessment were procured from Span Diagnostics, Surat, India.

Plant Extract Preparation

Gel parts from the 2 to 3 years old plant's leaves of *Aloe vera (L.)* were collected in June-July month of the year. About 500 mL of the gel part was mixed with 1 liter of methanol or, ethanol or, deionized water or, hydro-ethanol (40:60), or hydro-methanol (40:60) separately and left for two days. Using a rota evaporator, each of the prepared solvent-gel mixture was condensed and lyophilized. The lyophilized extracts were collected and stored at 8°C for future use in the experiment.

Experimental Design

The experimental framework was placed and sanctioned by the Animal Ethics Committee of our university, approval no. VU/IAEC-I/DG-1/3-15/19. The rules and regulations of the Govt. of India approved Committee for Control and Supervision of Experiments on Animals (CCSEA) were followed throughout the experiment. After delivery from the authorized vendor, rats (120 ± 10 g weight, two months age) were housed for ten days to acclimatize under controlled temperature (25 ± 2°C) and humidity (45–60%). Diabetes was developed by streptozotocin (STZ) injection through muscle for a single frequency, dosing 40 mg/kg of body weight. The control group was subjected to the injection of citric acid buffer, pH 4.5, dosing 1-mL/ kg of body weight, to match the level of physical stress with the diabetes group in connection with the injection, if any. On day seven of the STZ injection, a routine analysis of fasting blood glucose (BG) was done from the lateral tail vein. Streptozotocin-injected rats with fasting BG levels higher than 300 mg/dL but lower than 350 mg/dl were considered as diabetic model animals for this study. Six diabetic model rats were allotted for each specific solvent extract-treated group along with one untreated diabetes group of equal number of rats.

Vehicle-treated control group (VTCG)

Normoglycemic rats (70–80 mg/dl fasting BG) of the said group were charged with deionized water 5 mL/kg of BW through forceful gavage at 9 AM every day in an empty stomach.

Vehicle-treated diabetes group

Diabetic rats (300–350 mg/dl fasting BG) of vehicle-treated diabetes group (VTDG) were allowed for deionized water 5 mL/ kg of BW orally at 9 AM daily in an empty stomach.

Aqueous extract-treated diabetes group

Each rat of aqueous extract-treated diabetes group (AETDG) was charged with 200 mg aqueous extract of *Aloe vera (L.)* dissolved in 5 mL deionized water/kg of BW as previously.

Ethanol extract-treated diabetes group

Rats of ethanol extract-treated diabetes group (EETDG) were gone through oral treatment with 200 mg ethanol extract of *Aloe vera (L.)* dissolved in 5 mL deionized water/ kg of BW as the previous group.

Methanol extract-treated diabetes group

Oral administration of 200 mg methanol extract of *Aloe vera (L.)*/5 mL deionized water/kg of BW per day was done as the said group.

Hydro-ethanol extract-treated diabetes group

Diabetic rats of Hydro-ethanol extract-treated diabetes group (HEETDG) were treated with 200 mg hydro-ethanol extract of *Aloe vera (L.)* dissolved in 5 mL deionized water/ kg of BW/day orally at par previous condition.

Hydro-methanol extract-treated diabetes group (HMETDG)

Rats under this group were treated through oral gavage with 200 mg hydro-methanol extract of *Aloe vera (L.)*/5 mL deionized water/ kg of BW/day at 9 AM daily, as mentioned earlier.

The duration of each round of the seminiferous epithelium cycle in rats is approximately 12 to 14 days and for stage-VII spermatokinetic study, two rounds of the cell cycle are needed**.** So, the treatment was continued for 28 days to observe the accurate changes in germ cell kinetics. Food was supplied after 2 hours of treatment to avoid any kind of food-phytomolecule interaction. On the 29th day, all the experimental rats were sacrificed. Blood was collected and serum was separated from one part of the collected blood where rest part was used for the glycated hemoglobin study. Cauda epididymis was dissected from each animal and processed to assess sperm motility and count. The liver and one testis, skeletal muscle, and kidney were washed in normal saline to remove the surface blood and stored at -20°C to assess adopted biochemical parameters. Another testis of each animal was processed for histological observation after Bouin's fixation.

Fasting BG, HbA1c Levels and Hexokinase Activity

The lateral tail vein of rat was pricked with a needle to measure fasting BG by a one-touch glucometer (On

call plus, ACON Labs, USA). The whole blood of each rat was processed to measure the HbA1c level by kit (Meril Diagnostic, India). The said enzyme kinetics was assessed from the liver by a standard protocol.[12]

Alpha-glucosidase and Alpha-amylase Inhibitory Activities

Inhibitory activities of the said extracts on alpha-glucosidase (AG) and alpha-amylase (AA) were measured biochemically byrespective standard protocols with slight modification using acarbose as a positive control in both cases.[13] Acetone powder of rat intestine (Sigma, USA) was usedforinhibitory activity assessment of AG. About 50 µL of maltose was added to initiate the reaction in presence of several concentrations (20 µg–100 µg/mL) of *Aloe vera (L.)* extract or acarbose and it was terminated by boiling for 5 minutes. Using kit (Prism Diagnostic Pvt. Ltd., Mumbai, India), glucose level was measured.

In the assay mixture, 20 µL of AA solution and different concentrations of plant extracts (20 µg to 100 µg/mL) or acarbose were added separately and incubated for 10 minutes to assess AA inhibitory activity. An addition of 200 µL starch (1%) was allowed to initiate the reaction and 3,5-dinitrosalycylic acid was used to terminate the reaction.[13]

Serum Insulin and Testosterone

Rat insulin RayBio ELISA kit (USA) was used to measure serum insulin (SI) level, and the ELISA kit of Lilac Medicare (India) was used to quantify serum testosterone. [14,15]

Sperm Motility and Count

Cauda epididymis of each experimental rat was excised and flushed with normal saline to collect spermatozoa for assessing sperm count and motility by a standard procedure.[16]

Androgenic Key Enzyme

Kinetics of Δ5,3β-HSD and 17β-HSD enzymes were assessed from testicular tissue following standard methods.[15]

Markers of Oxidative Stress

Catalase and superoxide dismutase (SOD) activities were measured from the liver, skeletal muscle, testicles, and kidney by respective standard method. Ice-cold PBS (pH 7.4) was used as homogenizing media for liver, skeletal muscle, testicles, and kidney tissues. After homogenization, the homogenate was subjected to estimate thiobarbituric acid reactive substances (TBARS) level as per standard method. [15]

Serum Lipid Profile

Serum levels of lipoprotein of low-density (LDL-C), high-density (HDL-C) along with neutral lipid cum triglyceride (TG) and total quantity of cholesterol (TC) were measured using standard method (Span Diagnostic, India).[12]

Histo-architectural Study of Testis

Testis was sectioned at 5 µm thickness in microtome (Leica RM2245, Germany) after Bouin's fixation followed by dehydration and embedding.[15]

Study of Acute Toxicity and Toxicity profile

Rats were monitored continuously for 2 hours and in a gap of 1-hour for the next 6 hours to observe changes in their behavior, neurological, and autonomic profiles after administration of the said extracts throughout the experimental duration, if any.[13]

Kinetics of serum glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) were measured by a semi-auto analyzer as per the instruction provided in the respective kit (Meril Diagnostics, India).[15]

Data Analysis

The obtained data was statistically analyzed to know the significance level at $p < 0.05$ using analysis of variance (ANOVA) followed by multiple comparison student's two-tail t-test.[17]

Result And Discussion

Fasting BG and HbA1c both are the important markers for assessing the management of diabetes. The fasting BG and HbA1c levels in VTDG were increased significantly ($p < 0.05$) than VTCG. Elevated levels of fasting BG and HbA1c in the untreated diabetes group (UDG) confirmed the sustained state of diabetes. However, uninterrupted treatment with different solvent extracts of *Aloe vera (L.)* (DEAV) for four weeks corrected the fasting BG and HbA1c levels toward the VTCG. Among all the treated groups, HEETDG showed maximum efficacy in this concern (Fig. 1). Recovery of aforesaid parameters after DEAV treatment was strengthened by increased SI level and an elevation in insulin-dependent hexokinase enzyme activity that corrected the circulating glucose concentration towards the VTCG. This explanation is compatible with recently published research work by others using

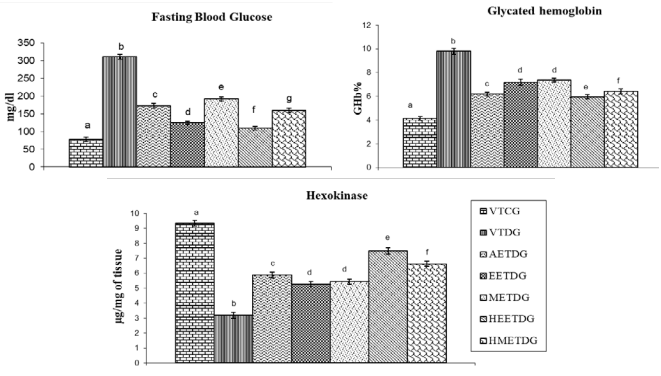

Fig. 1: Effect of DEAV on glycemic sensors. Statistical tool 'Analysis of Variance' followed by Multiple Comparison Student's two tail t-test was used. Each column represents Mean ± SEM, sample size (n = 6). Column with different superscripts (a,b,c,d,e,f,g) differ from one another significantly, $p < 0.05$.

Table 1: Inhibitory effect of DEAV on alpha-glucosidase and alpha-amylase enzyme activity

	IC_{50} values	
	Alpha-glucosidase (µg/mL)	*Alpha amylase (µg/mL)*
Aqueous extract of *Aloe vera (L.)*	77.19	74.35
Ethanol extract of *Aloe vera (L.)*	101.61	97.26
Methanol extract of *Aloe vera (L.)*	122.24	107.19
Hydro-ethanol extract of *Aloe vera (L.)*	57.35	61.50
Hydro-methanol extract of *Aloe vera (L.)*	64.2	69.33
Acarbose	43.54	56.43

another plant.[18,19] Individuals with diabetes had 1.5 and 2 times higher AG and AA activities, respectively.[20] Regularization of the said enzyme activities could be a key therapeutic tool to manage post-prandial hyperglycemia. An inhibitory concentration by 50 % of each extract was shown in Table 1. *Aloe vera (L.)* extracts showed potential AG and AA inhibitory activities, possibly through a competitive or non-competitive manner as reported by multiple studies in the same domain.[21,22] Hydro-ethanol (40:60) extract of *Aloe vera (L.)* possesses more efficacy in inhibiting the activities of said enzymes than other extracts considering acarbose as standard.

Enhanced glycation in diabetes itself may cause the production of highly reactive superoxide anions and hydroxyl radicals that deplete antioxidant enzyme activity (AEA).[23] Our results also showed a diminution in AEA at a significant level ($p < 0.05$) and a considerable rise in the concentration of TBARS, the lipid oxidation-peroxidation end metabolite, an important sensor of lipid metabolomics in diabetes. After treatment with DEAV, improvement in the said markers was noted towards the VTCG. Maximum recovery in the said markers was found in HEETDG in respect to other extracts (Table 3). Improvement of these markers may be explained as a downstream effect of the normalization of glycation rate by the phytomolecule(s) present in DEAV as similar results reported by other observers.[24] Another possibility may be the rectification in the testicular androgenesis process by phytomolecule(s) as genomic upregulation of antioxidant enzyme-encoded genes is under androgen regulation.[25] Such testicular androgenesis correction was supported here by improved serum testosterone levels in DEAV-treated diabetes groups.

Diabetes-induced hypo-testicular activity is multifactorial, and low insulin is one of the prime causes of it. Insulin binds to its receptor on Leydig cells, activating the signaling pathway of PI3K/Akt as messenger molecules, leading to glucose uptake that results in stimulation of androgenesis through activation of testicular steroidogenic key enzymes. The said androgenic regulating enzyme activity elevation has been confirmed here by the kinetics study of the relevant enzymes. The effect of insulin on Leydig cells for androgenesis is another possibility in this connection.[26] Insulin regulates the expression of transcription factors such as steroidogenic factor-1 (SF-1) and liver receptor homolog-1 (LRH-1) in Leydig cells, which regulates the expression of the genes encoding androgenic enzymes.[27,28] Low insulin and testosterone levels (TL) in serum and reduced key enzyme activities of androgen synthesis confirmed the hypo-functional state of testes in diabetes (Figs 2 and 3). These molecular effects are crucial for correcting normal testosterone production and male reproductive function in diabetes by phytomolecule(s) treatment**.** The decreased percentage of motile sperm and sperm count further proved the diabetes-associated hypo-functioning state of androgenesis and spermatogenesis. Amelioration was noted at a significant ($p < 0.05$) level after oral treatment of DEAV. The corrective efficacy of the hydro-ethanol extract of *Aloe vera (L.)* (HEEAV) was significantly higher in contrast to other extract-treated groups (Table 2).

The rectification of testicular androgenesis and gametogenesis in diabetes may be explained by the effective extract treatment that resettled insulin level followed by oxidative stress injury management through antioxidant enzyme activity correction and TBARS level resettlement towards the VTCG. This mechanism is compatible with existing literature using different plants in this line.[15]

Moreover, testicular histo-architecture supports the scenario of testicular disruption in diabetes and gradation of improvement after DEAV treatment against UDG. The VTCG shows normal size of the seminiferous tubule with normal sperm density and proper germ cell arrangement along the lumen of the seminiferous tubule in qualitative and semi-quantitative studies (Fig. 4A). A very minimum number of spermatozoa along the lumen and distortion in the shape of seminiferous tubule were noted in VTDG

Table 2: Remedial effect of DEAV on sperm motility and sperm count in STZ-induced diabetic rat

Spermiological sensors	VTCG	VTDG	AETDG	EETDG	METDG	HEETDG	HMETDG
Motile sperm (%)	76.33 ± 1.36^{a}	39.83 ± 2.48^{b}	58.33 ± 3.50^{c}	48.5 ± 2.5^{d}	53.5 ± 3.01^{e}	67.5 ± 2.88^{f}	61.66 ± 3.50^{g}
Sperm count (million/mL)	32.50 ± 0.61^{a}	11.25 ± 0.57^{b}	18.83 ± 0.47^{c}	16.83 ± 0.30^{d}	15.5 ± 0.42^{e}	26.5 ± 0.42^{f}	23.21 ± 0.36^{g}

Table 3: Effect of DEAV on oxidative stress parameters

Parameters	*Organs*	*VTCG*	*VTDG*	*AETDG*	*METDG*	*EETDG*	*HEETDG*	*HMETDG*
Catalase activity (nM H_2O_2 consumed/mg of tissue/min)	Liver	5.91 ± 0.27^a	2.88 ± 0.34^b	3.52 ± 0.19^c	3.48 ± 0.31^d	3.65 ± 0.27^e	4.76 ± 0.35^f	3.96 ± 0.20^g
	Testis	6.24 ± 0.14^a	1.89 ± 0.19^b	3.99 ± 0.12^c	3.07 ± 0.18^d	4.07 ± 0.19^e	5.02 ± 0.12^f	4.48 ± 0.15^g
	Kidney	6.53 ± 0.06^a	2.16 ± 0.03^b	3.94 ± 0.04^c	3.52 ± 0.06^d	4.04 ± 0.03^e	5.90 ± 0.055^f	4.23 ± 0.07^g
	Skeletal muscle	3.51 ± 0.06^a	1.27 ± 0.08^b	2.17 ± 0.03^c	1.97 ± 0.03^d	2.03 ± 0.09^e	3.17 ± 0.08^f	2.28 ± 0.04^g
SOD activity (Unit/mg of tissue)	Liver	5.39 ± 0.12^a	1.90 ± 0.12^b	3.04 ± 0.12^c	3.68 ± 0.12^d	3.32 ± 0.12^e	4.12 ± 0.12^f	3.93 ± 0.12^g
	Testis	4.85 ± 0.12^a	1.18 ± 0.12^b	3.64 ± 0.12^c	3.54 ± 0.12^d	3.15 ± 0.12^e	3.92 ± 0.12^f	3.69 ± 0.12^g
	Kidney	3.46 ± 0.04^a	1.28 ± 0.05^b	1.98 ± 0.05^c	2.18 ± 0.05^d	2.33 ± 0.05^e	2.88 ± 0.04^f	2.30 ± 0.04^g
	Skeletal muscle	3.49 ± 0.034^a	0.96 ± 0.08^b	1.44 ± 0.02^c	1.53 ± 0.03^d	1.71 ± 0.03^e	2.96 ± 0.03^f	2.66 ± 0.03^g
TBARS level (nM/mg of tissue)	Liver	37.37 ± 2.59^a	87.24 ± 2.97^b	67.94 ± 2.57^c	58.17 ± 2.55^d	65.29 ± 2.36^e	47.26 ± 1.71^f	53.84 ± 1.62^g
	Testis	30.43 ± 2.77^a	75.38 ± 2.72^b	57.77 ± 2.92^c	54.74 ± 2.94^d	52.16 ± 2.85^d	41.54 ± 2.60^e	50.25 ± 2.46^d
	Kidney	20.05 ± 0.88^a	72.90 ± 1.87^b	50.40 ± 0.74^c	55.41 ± 0.74^d	53.40 ± 0.93^d	26.60 ± 0.41^e	37.60 ± 0.41^f
	Skeletal muscle	13.88 ± 0.49^a	50.66 ± 1.15^b	38.45 ± 0.46^c	37.51 ± 0.46^d	33.59 ± 0.46^e	18.92 ± 0.39^f	22.92 ± 0.39^g

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

Table 4: Ameliorative effect of DEAV on serum lipid and toxicity profile

Serum parameters	VTCG	VTDG	AETDG	EETDG	METDG	HEETDG	HMETDG
TC (mg/dl)	47.19 ± 3.2^a	131.05 ± 2.06^b	83.43 ± 2.90^c	96.21 ± 1.51^d	101.20 ± 2.22^e	61.12 ± 1.65^f	69.65 ± 2.02^g
TG (mg/dl)	73.88 ± 2.51^a	173.29 ± 2.36^b	109.62 ± 3.30^c	127.23 ± 1.28^d	139.03 ± 2.51^e	88.19 ± 2.14^f	93.39 ± 2.29^f
LDL-C (mg/dl)	21.04 ± 1.12^a	60.22 ± 3.01^b	38.56 ± 2.03^c	43.24 ± 3.01^d	48.64 ± 3.23^d	27.04 ± 1.57^e	31.64 ± 2.45^f
HDL-C (mg/dl)	63.23 ± 1.23^a	26.31 ± 2.11^b	40.15 ± 1.64^c	39.19 ± 1.12^c	32.33 ± 1.69^d	53.68 ± 3.32^e	46.15 ± 1.06^f
SGOT (Unit/L)	0.69 ± 0.16^a	3.98 ± 0.12^b	1.44 ± 0.13^c	2.57 ± 0.15^d	2.55 ± 0.12^d	1.07 ± 0.12^e	1.49 ± 0.15^f
SGPT (Unit/L)	0.57 ± 0.12^a	3.95 ± 0.12^b	1.51 ± 0.13^c	2.57 ± 0.14^d	2.74 ± 0.14^e	0.93 ± 0.13^f	1.06 ± 0.15^g

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

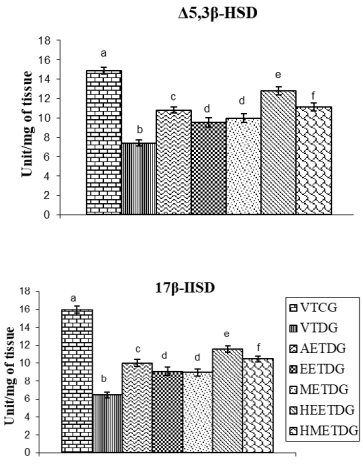

Fig. 2: Correction in the androgenic key enzyme activities after 28 days of treatment with DEAV. Statistical tool 'Analysis of Variance' followed by Multiple Comparison Student's two tail t-test was used. Each column represents Mean ± SEM, sample size (n) = 6. Column with different superscripts (a,b,c,d,e,f) differ from one another significantly, $p < 0.05$.

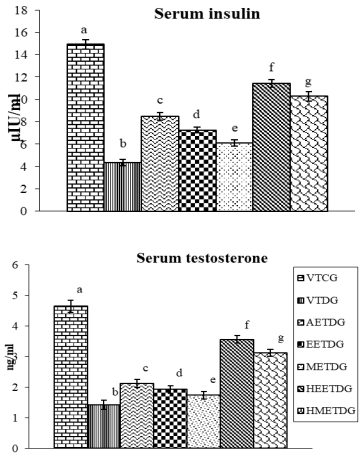

Fig. 3: Ameliorative effect of DEAV on serum insulin and testosterone levels. Statistical tool 'Analysis of Variance' followed by Multiple Comparison Student's two tail t-test was used. Each column represents Mean ± SEM, sample size (n) = 6. Column with different superscripts (a,b,c,d,e,f,g) differ from one another significantly, $p < 0.05$

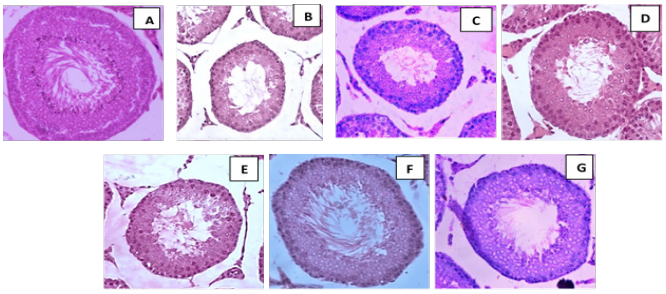

Fig. 4: Representative histo-architecture of testis covering germ cell population and physical shape of the seminiferous tubule (hematoxylin & eosin stain, 400X)

compared to the VTCG (Fig. 4-B). Fig. 4C-G showed the recovery in of germ cell arrangement and distortion of seminiferous tubular structure in aqueous, ethanol, methanol, hydro-ethanol (40:60), and hydro-methanol (40:60) extract-treated diabetes groups respectively. Concomitant correction was noted after each extract treatment but maximally noted in HEETDG.

Previous findings have already proved the association between hyperlipidemia and vascular complications in diabetes.[12] The LDL-C, triglyceride, and cholesterol levels were higher, and HDL-C level was lower in VTDG than the VTCG. The serum toxicity profile showed higher activity of GOT and GPT in VTDG than the VTCG. The aforementioned parameters were rectified significantly ($p < 0.05$) in *Aloe vera (L.)* extract-treated diabetes groups where the HEETDG showed the highest percentage of recovery than other extracts (Table 4). The phytomolecule(s) present in DEAV may have statins-like effects that attenuate the serum lipid profile towards the VTCG. [29] Significant ($p < 0.05$) recovery of serum GOT and GPT activities in DEAV-treated diabetes groups denotes that the extract is not toxic, moreover it has efficient detoxification properties. This was also supported by an acute toxicity study where no such abnormalities in terms of changes in behavior, sleep, walking, and response to human touch were observed in extract-treated diabetes groups. Tremors, diarrhea, salivation, and death were not noted throughout the experiment in DEAV-treated diabetes groups. However, all the signs except salivation and death were noted in VTDG.

Conclusion

The obtained results reflect that the phytomolecule(s) in the leaf gel extract of *Aloe vera (L.)* can manage diabetes and the diabetes-linked hypo-functional state of testis. Among all the solvents used for the extraction purpose, high recovery percentage was noted in the hydro-ethanol (40:60) extract-treated diabetes group. As different extracts of the plant materials contain various bioactive compounds [30] so, it may be stated that phytomolecule(s) involved in this correction are more abundant in hydro-ethanol (40:60) extract than others. Further study regarding the isolation of particular phytomolecule(s) for such rectification is needed in connection with effective and promising herbal drug development against diabetes and testicular hypo-function co-morbidity.

Acknowledgement

The authors are grateful to the Department of Science & Technology, Govt of India (GOI) for funding this project (Project no: DST/INSPIRE Fellowship/2018/IF180939).

References

1. Öztaş E, Yılmaz TE, Güzel E, Sezer Z, Okyar A, Özhan G. Gliclazide alone or in combination with atorvastatin ameliorated reproductive damage in streptozotocin-induced type 2 diabetic male rats. Saudi Pharmaceutical Journal. 2019; 27(3):422–31. Available from: https://doi.org/10.1016/j.jsps.2019.01.003
2. Heeba GH, Hamza AA. Rosuvastatin ameliorates diabetes-induced reproductive damage via suppression of oxidative stress, inflammatory and apoptotic pathways in male rats. Life Science. 2015; 141(1):13–9. Available from: https://doi.org/10.1016/j.lfs.2015.09.015
3. Rabbani SI, Devi K, Khanam S. Inhibitory effect of glimepiride on nicotinamide-streptozotocin induced nuclear damages and sperm abnormality in diabetic Wistar rats. Indian Journal of Experimental Biology. 2009; 47(10):804–10.
4. La Vignera S, Calogero AE, Condorelli R, Lanzafame F, Giammusso B, Vicari E. Andrological characterization of the patient with diabetes mellitus. Minerva Endocrinology. 2009; 34(1):1–9.
5. Mohasseb M, Ebied S, Yehia MA, Hussein N. Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. Journal of Physiology and Biochemistry. 2011; 67(2):185–94. Available from: https://doi.org/10.1007/s13105-010-0062-2
6. Kanter M, Aktas C, Erboga M. Curcumin attenuates testicular damage, apoptotic germ cell death, and oxidative stress in streptozotocin-induced diabetic rats. Molecular Nutrition & Food Research. 2013; 57(9):1578–85. Available from: https://doi.org/10.1002/mnfr.201200170
7. Ghanbari E, Nejati V, Najafi G, Khazaei M, Babaei M. Study on the effect of royal jelly on reproductive parameters in streptozotocin-induced diabetic rats. International Journal of Fertility & Sterility. 2015; 9(1):113-20. Available from: https://doi.org/10.22074/ijfs.2015.4215
8. Mao CF, Sudirman S, Lee CC, Tsou D, Kong ZL. *Echinacea purpurea* ethanol extract improves male reproductive dysfunction with streptozotocin-nicotinamide-induced diabetic rats. Frontiers in Veterinary Science. 2021; 8:1-11. Available from: https://doi.org/10.3389/fvets.2021.651286
9. Majumder R, Das CK, Mandal M. Lead bioactive compounds of *Aloe vera* as potential anticancer agent. Pharmacological Research. 2019; 148:1-35. Available from: https://doi.org/10.1016/j.phrs.2019.104416
10. Kumar R, Singh AK, Gupta A, Bishayee A, Pandey AK. Therapeutic potential of *Aloe vera*-A miracle gift of nature. Phytomedicine. 2019; 60:1- 46 . Available from: https://doi.org/10.1016/j.phymed.2019.152996
11. Suksomboon N, Poolsup N, Punthanitisarn S. Effect of *Aloe vera* on glycaemic control in prediabetes and type 2 diabetes: A systematic review and meta-analysis. Journal of Clinical Pharmacy and Therapeutics. 2016; 41:180-8. Available from: https://doi.org/10.1111/jcpt.12382
12. Bera TK, Ali KM, Jana K, Ghosh A, Ghosh D. Protective effect of aqueous extract of seed of *Psoralea corylifolia* (Somraji) and seed of *Trigonella foenum-graecum L.* (Methi) in streptozotocin-induced

diabetic rat: A comparative evaluation. Pharmacognosy Research. 2013; 5(4):277-85. Available from: https://doi.org/10.4103/0974-8490.118840
13. De D, Bera TK, Ali KM, Mandal S, Barik B, Ghosh D. Inhibitory effects of n-hexane fraction from hydro-methanolic (2: 3) extract on α-amylase and α-glucosidase activities of sepal of *Salmalia malabarica*: An approach through in vitro study. Biomarkers and Genomic Medicine. 2013; 5(1-2):48-53. Available from: https://doi.org/10.1016/j.gmbhs.2013.04.003
14. Bürgi W, Briner M, Franken N, Kessler AC. One-step sandwich enzyme immunoassay for insulin using monoclonal antibodies. Clinical Biochemistry. 1988; 21(5):311–4. Available from: https://doi.org/10.1016/s0009-9120(88)80087-0
15. 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 Food Biochemistry. 2019; 43(5):1-13. Available from: https://doi.org/10.1111/jfbc.12829
16. World Health Organization. WHO laboratory manual for the examination and processing of human semen. Cambridge University Press, 2021.
17. Sokal RR, Rohle FJ. Biometry. WH Freeman and Company, 1997.
18. Gothandam K, Ganesan VS, Ayyasamy T, Ramalingam S. Antioxidant potential of theaflavin ameliorates the activities of key enzymes of glucose metabolism in high fat diet and streptozotocin - induced diabetic rats. Redox Report. 2019; 24(1):41-50. Available from: https://doi.org/10.1080/13510002.2019
19. Ara F, Tripathy A, Ghosh P, Ghosh D. Correction of glycemic and oxidative stress sensors in streptozotocin induced diabetic rat model: Impact of dose dependent study of n-butanol solvent fraction of hydro-methanol extract of banana flower (*Musa balbisiana*). Journal of Food Biochemistry. 2020; 44(10):1-13. Available from: https://doi.org/10.1111/jfbc.13386
20. Yin Z, Zhang W, Feng F, Zhang Y, Kang W. α-Glucosidase inhibitors isolated from medicinal plants. Food science and human wellness. 2014; 3 (3-4):136-74. Available from: https://doi.org/10.1016/j.fshw.2014.11.003
21. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida Benth* leaf. BioMed Research International. 2013; 2013(3):1-6. Available from: https://doi.org/10.1155/2013/527570
22. Feng Y, Nan H, Zhou H, Xi P, Li B. Mechanism of inhibition of α-glucosidase activity by bavachalcone. Food Science and Technology. 2022; 42:1-6. Available from: https://doi.org/10.1590/fst.123421
23. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015; 5(1):194-222. Available from: https://doi.org/10.3390/biom5010194
24. Meenatchi P, Purushothaman A, Maneemegalai S. Antioxidant, antiglycation and insulinotrophic properties of *Coccinia grandis* (L.) in vitro: Possible role in prevention of diabetic complications. Journal of Traditional and Complementary Medicine. 2017; 7(1):54-64. Available from: https://doi.org/10.1016/j.jtcme.2016.01.002
25. Kolasa-Wołosiuk A, Tarnowski M, Baranowska-Bosiacka I, Chlubek D, Wiszniewska B. Antioxidant enzyme expression of mRNA and protein in the epididymis of finasteride-treated male rat offspring during postnatal development. Achieves of Medical Sciences. 2019; 15(3):797-810. Available from: https://doi.org/10.5114/aoms.2017.68528
26. Ni FD, Hao SL, Yang WX. Molecular insights into hormone regulation via signaling pathways in Sertoli cells: With discussion on infertility and testicular tumor. Gene. 2020; 753:1-13. Available from: https://doi.org/10.1016/j.gene.2020.144812
27. Kinyua AW, Doan KV, Yang DJ, Huynh MKQ, Choi YH, Shin DM, Kim KW. Insulin regulates adrenal steroidogenesis by stabilizing SF-1 activity. Scientific Reports. 2018; 8(1):1-9. Available from: https://doi.org/10.1038/s41598-018-23298-2
28. Ahn SW, Gang GT, Kim YD, Ahn RS, Harris RA, Lee CH, Choi HS. Insulin directly regulates steroidogenesis via induction of the orphan nuclear receptor DAX-1 in testicular Leydig cells. Journal of Biological Chemistry. 2013; 288(22):15937-46. Available from: https://doi.org/10.1074/jbc.M113.451773
29. Attanayake AP, Jayatilaka K A, Mudduwa LK, Pathirana C. Biochemical and histological evaluation of three selected medicinal plant extracts of Sri Lankan origin on dyslipidemia and oxidative stress in alloxan induced diabetic rats. Journal of Botany. 2018; 2018:1-8. Available from: https://doi.org/10.1155/2018/4204519
30. Truong DH, Nguyen DH, Ta NT, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. Journal of Food Quality. 2019; 2019:1-9. Available from: https://doi.org/10.1155/2019/8178294

HOW TO CITE THIS ARTICLE: 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 Int. J. Pharm. Sci. Drug Res. 2024;16(3):458-464. **DOI:** 10.25004/IJPSDR.2024.160319