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Urodynamic parameters in stress-induced incontinence in female diabetic rats: Possible beneficial role of Abscisic acid

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- Diabetes Mellitus (DM)
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Abstract

Diabetes mellitus (DM) is a widespread disease expected to affect 693 million by 2045. A significant but often overlooked complication of type 2 DM in the elderly is stress urinary incontinence (SUI), which impairs their quality of life. Abscisic acid (ABA), a plant phytohormone, has been found in human cells such as granulocytes and pancreatic beta cells. This study aimed to explore the protective and therapeutic effects of ABA on SUI in female diabetic rats. Forty rats were assigned to five groups, including control, VD-induced SUI, VD+DM, VD+ABA, and VD+DM+ABA groups. ABA was administered orally for 8 weeks, with additional treatment post-VD in some groups. We assessed glucose levels (HbA1c, insulin), oxidative stress (LDH), renal markers (urea, creatinine), and SUI (ALPP). Histological and immunohistochemical analysis evaluated urethral tissue, caspase-3, and LC3 expression. Results showed ABA improved glucose regulation, reduced LDH, urea, and creatinine levels, and increased ALPP. Histology indicated less inflammation and fibrosis in ABA-treated groups. Caspase-3 and LC3 expression were modulated favorably by ABA. Thus, ABA showed both prophylactic and therapeutic effects against SUI in diabetic rats through its anti-inflammatory and antiapoptotic actions

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1. Introduction

Diabetes mellitus (DM) is a common and fast-growing disease globally, estimated to affect 693 million adults by 2045, more than a fifty percent increase from 2017 **(1)**. Diabetes is typically divided into an early-onset, insulin-dependent form (type 1 DM; T1DM) and a late- onset, non-insulin-dependent form type 2 DM (T2DM) **(2)**. Diabetic cases often complain of several adverse events, which include macrovascular and microvascular diseases **(3)**.

Urinary incontinence (UI) is a common but under-recognized adverse event of T2DM for older adults aged 65 years **(4; 5)**. While UI wouldn't be considered a fatal adverse event of DM, it has a devastating effect on the human health, function, and quality of life of the elderly as a result; they desire to avoid living in community **(5)**. In the Middle East, about 40% of females older than 65 complain of UI

(6). UI is described as the complaint of involuntary leaking of urine **(7)**. Much research demonstrates a correlation between DM and UI, but studies assessing DM as a predisposing factor for UI have conflicting results, though most of them find that diabetic females have a higher risk **(8)**. The number of cases with DM-related urinary adverse events has been considerably increasing in the past few decades due to the growing prevalence of DM **(9)**. The lower urinary tract symptoms (LUTS), that could result, are called "diabetic cystopathy" or "diabetic bladder dysfunction" (DBD) **(10)**. DBD could describe a broad spectrum of LUTS, such as detrusor overactivity (DO), and impairment of bladder contractility, areflexic bladder, urine retention, and incontinence. It is accompanied by diminished sensation to filling and increased bladder capacity, making the phenotype heterogenous **(11)**.

Abscisic acid (ABA) is a phytohormone controlling vital physiologic process in plants **(12)**. In recent years, ABA has been recognized in mammalian plasma and a lot of cell kinds which include human granulocytes and pancreatic beta cells **(13)**. ABA, at Nanomolar concentrations, improves insulin release as a result of glucose in rat insulinoma cells, and human pancreatic islets **(14)**. In humans, ABA naturally arises from dietary sources and endogenous formation via the

carotenoid biogenesis pathway **(15)**. ABA was first discovered in the brain of pigs and rats **(16)**. Indirect evidence that ABA was formed endogenously was supported by the fact that animals fed a synthetic, ABA-free food had ABA levels that were even greater than those of controls on a vegetable diet **(16)**. So far, a newer thought demonstrates that ABA is in fact an endogenous signalling molecule: there was a significant increase in plasma ABA levels among normal subjects following an (ABA-free) glucose load **(17)**. In contrast, this observation suggests ABA as being endogenously synthesized; in addition, it supports ABA role in the physiologic response to glucose intake, a role until then shared by insulin and by the incretin glucagon-like peptide- 1 (GLP-1) **(17)**. In addition, with regard to normal individuals,

the use of two various dosages of ABA added to glucose drink formed, for the higher one, a significant reduction in PPG and insulin values (around −25%) whereas, for the lower dosage, a significant decrease in postprandial insulin concentration (around −15%) with a dose-response lowering for the two ABA amounts **(18)**. The lower dosage diminished, but not in a significant manner, PPG values by about 14% (19). Interestingly, emerging research suggests that ABA's glucose-lowering effects may also involve anti-inflammatory and antioxidant pathways. Chronic hyperglycemia induces the generation of free radicals and inflammation, which further impair insulin signaling and beta-cell function **(19)**. ABA has been shown to modulate cytokine profiles and reduce oxidative stress markers in experimental models **(20)**. Thus, part of its beneficial action on glucose homeostasis may be indirect, via mitigation of systemic inflammation and enhancement of insulin signaling pathways. Thus, the aim of the present study to investigate whether ABA has preventive and/or therapeutic role in SUI in female diabetic Sprague-Dawley rats.

2. Materials and methods

2.1. Experimental animals

This study was conducted on 40 adult female Sprague-Dawley rats weighing 200-250 grams. Animals were bred and housed in the animal house of MERC, Mansoura faculty of medicine, Mansoura University. The study design was approved by Mansoura faculty of medicine committee of animal care and ethics and given code number MU-AUC (MED.MS.23.02.7).

2.2. Study design

Rats were divided into five groups; each group included 8 rats:

Group I (control); in this group rats were housed normally.

Group II (VD only); in this group, only SUI was induced via vaginal dilatation (VD) model.

Group III (VD+DM); in this group, first diabetes had been induced, then VD was done 8 weeks later. Group IV (VD+ABA); in this group the rats had received abscisic acid (ABA) at a dose of (1mg/kg/day) by oral gavage for 8 weeks, then VD was done, after that, rats continued to receive ABA for another week.

Group V (DM+VD+ABA); in this group, first DM had been induced, then ABA was given at a dose of (1mg/kg/day) by oral gavage for 8 weeks, after that, VD was done and rats continued to receive ABA for another week.

2.3. Chemicals

Abscisic acid vials were obtained from Bio Basic Inc, Canada through their Egyptian distributor Gene Tech in the form of white powder in 100 mg packages (C 15H 2004, Purity >98%, MW: 264.3). Streptozotocin (STZ) used for induction of diabetes was brought from Sigma Aldrich (SO130- 1G).

2.4. Induction of diabetes mellitus:

Diabetes was caused by injection of a single dose of freshly prepared STZ (50 mg/kg) intraperitoneally. After 72 hours of injection, random blood glucose level was estimated to assess induction of DM.

2.5. Induction of SUI by vaginal dilatation (VD) model:

The rats were weighed and then anaesthetized with ketamine (75mg/kg) and xylazine (10mg/kg) respectively **(21)** and given intraperitoneally in the supine position with the limbs of the rat connected to the table and pulled towards the cage top. After that, a eight-fr foley catheters were introduced into the vagina, and the balloons were inflated with five ml of sterile saline. The labia majora were sutured in a figure –eight pattern with five-zero surgical needles to avoid the ejection of the balloon. The balloon was placed to prevent close contact with the table surface and left to hang from the bottom of the cage; balloon was withdrawn after eight hours.

2.6. Measurement of ALPP to assess SUI

Rats were anaesthetized with ketamine (75mg/kg) and xylazine (10 mg/kg) respectively given intraperitoneally **(21)**. The urethra catheterization was performed by using three-way (24G) cannula for saline infusion and it was attached to the physiologic pressure transducer to record the abdominal leak point pressure (ALPP). Alterations in ALPP were recorded by a computer with ADINSTRUMENTS power lab 4/30.

The utilized apparatus is composed of Bridge Amp. attached to a Power Lab 4/30 recording unit with its high-grade isotonic transducer and additional analysis with chart seven software.

2.7. Biochemical analysis:

- The following parameters were measured:

a) Assessment of glucose homeostasis via measuring HbA1c and serum insulin

b) Measurement of LDH as an oxidative stress marker

c) Assessment of kidney function via measuring serum urea and creatinine.

2.8. Histopathological examination of urethral specimens

A part from urethra was fixed in ten percent formaldehyde then maintained in paraffin for histopathological assessment. Then, the paraffin blocks were dissected into 7-10µm thick sections. These specimens were stained by H&E for evaluation of histopathological change, and by Masson trichrome stain for assessment of fibrosis, tissue damage, and architectural changes in urethra.

2.9. Histopathological scoring for urethral wall changes among different groups by H&E and Masson's Trichrome

Data of urethral lumen diameter was analyzed using one way Analysis of Variance (ANOVA) followed by Tukey's test to compare all means. Inflammation scores in urethral wall were statistically analysed using Kruskal Wallis One Way ANOVA on Ranks with all pairwise multiple comparison procedures done by Dunn's Method. Different letters mean significant when ($P<0.05$).

2.10. Immunohistochemical study of apoptotic marker caspase-3 and autophagy marker LC-3 Specimens were deparaffinized in xylene rehydrated through descending alcohol concentrations, and rinsed in distilled water. Antigen retrieval was conducted using citrate buffer (10 mM, pH 6.0) in a water bath at 95°C for 20–30 minutes, followed by

gradual cooling at 22°C and washing with phosphate-buffered saline (PBS). Endogenous peroxidase activity was quenched using 3% hydrogen peroxide (H2O2), and non- specific binding was blocked by incubation of the slides with five percent normal goat serum for thirty minutes at 22°C. Incubation was conducted overnight at 4°C with a primary rabbit anti-cleaved caspase-3 antibody (Ab) at an optimized dilution. After thorough washing in PBS, slides were incubated with an HRP-conjugated secondary Ab for 30 minutes. Visualization was achieved using 3,3'-diaminobenzidine (DAB) chromogen, producing a brown reaction product at the site of caspase-3 expression. Counterstaining was performed with hematoxylin, followed by dehydration, clearing in xylene, and mounting with DPX mounting medium.

Urethral samples were fixed in ten per cent neutral- buffered formalin for one–two days, dehydrated through ascending ethyl alcohol concentrations, cleared in xylene, and embedded in paraffin. Sections of 4–5 µm thickness were cut using a rotary microtome and mounted on positively charged glass slides, then dried overnight at 37°C. Deparaffinization was conducted using xylene, followed by rehydration through graded ethanol series and rinsing in distilled water. Antigen retrieval was conducted in 10 mM citrate buffer (pH 6) by heating in a water bath at 95°C for 20–30 minutes. After cooling at 22°C and rinsing with PBS, endogenous peroxidase activity was blocked using three percent H_2O_2 for ten minutes. Non- specific protein binding was reduced by incubating the sections with 5% normal goat serum for thirty minutes. Slides were then incubated overnight at 4°C with a primary Ab specific for LC3 (typically rabbit anti-LC3, at a dilution optimized based on the manufacturer's instructions). Following PBS washes, an HRP-conjugated secondary Ab was applied for 30 minutes at 22°C. The antigen-Ab complex was visualized using DAB chromogen, resulting in a brown precipitate indicative of LC3 expression. Sections were counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted using DPX mounting medium.

2.11. Morphometric analysis of apoptotic marker caspase-3 and autophagy marker LC-3 Bars are means ± SE. Caspase-3 and LC-3 IHC scores in urethral sections were statistically analyzed using Kruskal-Wallis One Way ANOVA on Ranks with all pairwise multiple comparison approaches conducted by Dunn's Method. Different letters mean significant when ($P < 0.05$)

2.21. Statistical analysis

Our study was conducted to investigate the potential beneficial role of abscisic acid on stress-induced urinary incontinence in female diabetic rats. The data were collected, tabulated and analyzed by SPSS, version 27; SPSS Inc., Chicago, Illinois, USA on an IBM-compatible computer.

Data were expressed, as mean and SD. ANOVA test was utilized to compare groups with quantitative variables, and significance values have been adjusted by the Bonferroni correction for multiple test

1. Results

1.1. Effect of induction of DM and administration of ABA on HbA1c and serum insulin among the different groups

Table 1 shows that induction of DM led to a significant increase in HbA1c levels in group 3 (VD+DM) compared to control group.

Administration of abscisic acid reduced HbA1c levels in group 5 (VD+DM+ABA) but is not statistically significant compared to group 3. Moreover, HbA1c levels remained elevated in group 5, which was statistically significant compared with control group. Insignificant changes were noticed in HbA1c among non-diabetic groups; groups 1,2 and 4.

Table 1 shows that induction of DM resulted in a significant reduction in serum insulin level in group 3 (VD+DM) compared to control group. Group 5 (VD+DM+ABA) showed significant elevation in serum insulin level which is significant compared to group 3 (VD+DM).

Despite this improvement, insulin level in group 5 remained significantly lower than that of control group. Insignificant changes in serum insulin levels were noticed among non-diabetic groups; groups 1,2 and 4.

1.2. Effect of induction of DM, SUI and ABA administration on serum LDH (oxidative stress marker):

Table 1 demonstrates that the induction of DM in conjunction with SUI in group 3 (VD+DM), resulted in a significant increase in serum lactate dehydrogenase (LDH) levels compared with the control group. Rats in group 2 (VD only) also exhibited a significant elevation in serum LDH levels relative to controls. However, comparison between group 3 (VD+DM) and group 2 (VD only) revealed a significantly higher serum LDH levels in group 3, suggesting that the combined insults of DM and SUI induced more severe tissue injury than SUI alone which is reflected by the significant elevation of LDH among all groups.

Oral administration of ABA caused a significant reduction in serum LDH levels in group 4 (VD+ABA) compared to group 2 (VD only). Furthermore, ABA administration in group 5 (VD+DM+ABA) significantly decreased serum LDH levels compared to group 3 (VD+DM). Although ABA administration improved LDH levels in groups 4 and 5, the serum LDH levels in those groups remained significantly elevated compared to the control group.

1.3. Effect of induction of DM, SUI and ABA administration on renal function (serum urea and creatinine):

Table 1 shows that the induction of DM in combination with SUI (Group 3) caused a significant increase in serum urea and creatinine levels compared to control group. In contrast, no significant differences were observed in serum urea and creatinine levels between Group 1 and both Group 2 and Group 4 (VD+ABA).

Administration of ABA was associated with a significant reduction in both serum urea and creatinine levels in group 5 (VD+DM+ABA) compared to group 3 (VD+DM). Despite this improvement, serum urea and creatinine levels in Group 5 (VD+DM+ABA) remained significantly elevated compared to the control group.

1.4. Effect of induction of DM, SUI and ABA administration on abdominal leak point pressure (ALPP):

Table 1 demonstrates that the induction of DM and SUI in group 3 (VD+DM) resulted in a significant reduction in ALPP compared to the control group. Rats in group 2 (VD only) also exhibited a significant reduction in ALPP in comparison with the controls. However, ALPP is significantly reduced in group 3 compared to group 2.

Administration of ABA led to significant improvement in ALPP in group 4 (VD+ABA) compared to group 2 (VD only). Moreover, ABA administration in group 5 (VD+DM+ABA) improved the ALPP in a significant manner compared to group 3 (VD+DM). Although ALPP has improved in both groups that received ABA; groups 4 and 5. There was still significant decrease in ALPP in both of them compared to control rats.

1.5. Histopathological examination and scoring from urethral specimens among different groups following induction of DM, SUI and ABA administration by H&E and Masson Trichrome.

- By H&E:

Figure 1 shows normal morphology of urethral wall in control group consisting of numerous layers, beginning with the urothelium, then the underlying lamina propria, a layer of smooth muscle, and a thick layer of striated muscle **(1A)**. While in group where SUI was induced by VD, the urethra shows narrowing of urethral lumen, marked fibrosis with mild periurethral inflammation **(1B)**. In group 3 where both DM and SUI were induced, urethra shows marked narrowing of urethral lumen, moderate periurethral inflammation, and severe fibrosis **(1C)**. On the other side, urethra from group 4 (VD+ABA) shows dilated urethral lumen, marked fibrosis with mild periurethral inflammation **(1D)**. Urethra from group 5 (VD+DM+ABA) shows narrow urethral lumen, mild periurethral inflammation **(1E)**.

- By Masson Trichrome:

Figure 2 shows microscopic picture of urethral sections, from control group, there is neither fibrosis nor excess bluish collagen deposition **(2A)**.

Urethra from Group 2 (VD only) shows excess bluish collagen deposition **(2B)**. Urethra from group 3 (VD+DM) shows marked bluish collagen deposition **(2C)**. Urethra from group 4 (VD+ABA) shows markedly decreased bluish collagen deposition **(2D)**. Urethra from group 5 (VD+DM+ABA) shows mild periurethral bluish collagen deposition **(2E)**.

1.6. Immunohistochemistry and morphometric analysis for expression of apoptotic marker caspase-3 and autophagy marker LC-3 among the different groups:

Figure 3 shows microscopic picture of urethral sections, from control group where there is negative expression for caspase-3 **(3A)**. Urethra from group 2 (VD only) shows positive brown expression for caspase-3 in urothelium **(3B)**. Urethra from group 3 (VD+DM) shows the most elevated positive

brown expression for caspase- 3 in urothelium among the 5 groups **(3C)**. Urethra from group 4 (VD+ABA) shows little expression for caspase-3 compared to group 2 (VD only) **(3D)**. Urethra from group 5 (VD+DM+ABA) shows less positive brown expression for caspase-3 in urothelium compared to group 3 (VD+DM+ABA) **(3E)**.

Figure 4 shows microscopic picture of urethral sections, from control group where there is negative expression for LC-3 **(4A)**. Urethra from group 2 (VD only) shows marked positive brown expression for LC-3 in urothelium **(4B)**. Urethra from group 3 (VD+DM) shows the most prominent expression for LC- 3 in urothelium among the 5 groups **(4C)**. Urethra from group 4 (VD+ABA) shows little expression for LC-3 compared to group 2 (VD only) **(4D)**. Urethra from group 5 (VD+DM+ABA) shows less positive expression for LC-3 in urothelium compared to group 3 (VD+DM+ABA) **(4E**).

Beneficial role of ABA in DM and SUI

Table (1): Effect of induction of DM, SUI and administration of ABA on HbA1c, serum insulin, LDH, urea and creatinine and ALPP among the different groups (G1: control group, G2: VD only group, G3: VD+DM group, G4: VD+ABA group, G5: VD+DM+ABA group; (a) significant to G1, (b) significant to G2, (c) significant to G3, (d) significant to G4).

	G1: normal N=8	**G2: VD only N=8**	**G3: VD+DM N=8**	**G4: VD+ABA N=8**	**G5: VD+DM+ABA N=8**
HbA1c	4.94 ± 0.56	5.96 ± 0.17	9.14 ± 0.77 ^{a***}	5.56 ± 0.28 ^{c}***	8.86 ± 0.64 ^{abd}***
Serum insulin	28.75 ± 8.07	28.10 ± 6.80	3.12 ± 0.080 ^{ab}***	26.19 ± 7.32 ^{c}***	5.56 ± 1.46 ^{abcd}***
Serum LDH	314 ± 56	631 ± 87 ^{a}***	969 ± 79 ^{ab}***	588 ± 28 ^{abc}***	766 ± 53 **acd***
Serum urea	29 ± 8	38 ± 5	58 ± 13 **ab***	33 ± 3 ^{c}***	40 ± 3 **c***
Serum creatinine	0.80 ± 0.11	0.79 ± 0.09	2.07 ± 0.66 ^{ab}***	0.78 ± 0.15 ^{c}***	1.23 ± 0.27 ^{c}***
ALPP	1.25 ± 0.174	0.683 ± 0.176 ^{a}***	0.435 ± 0.06 ^{a}***	0.815 ± 0.07 ^{abc}***	0.635 ± 0.112 ^{abcd}***

***Significance at p-value <0.001

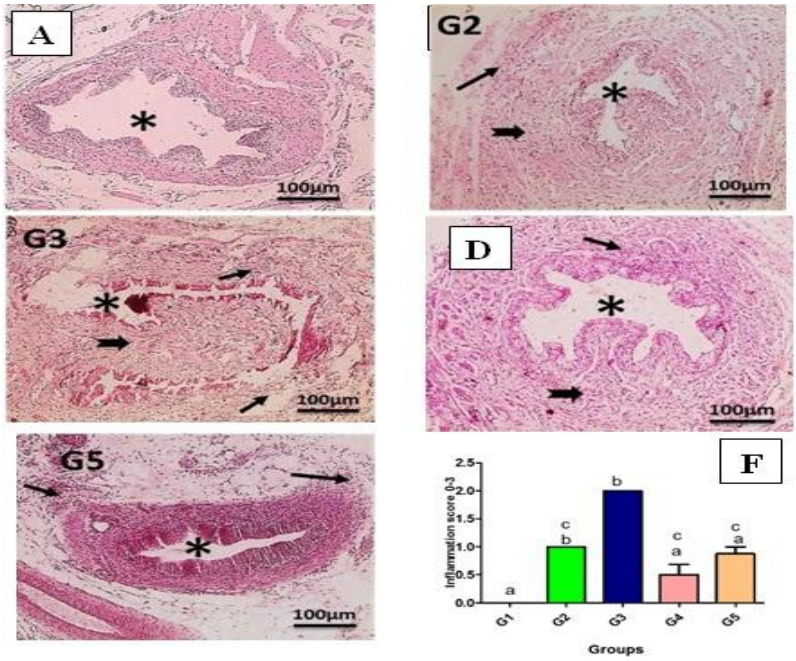

Figure (1): (A-E): Microscopic picture of urethral sections from **A** (control group) shows normal morphology consisting of several layers, starting with the urothelium, then the underlying lamina propria, a layer of smooth muscle, and a thick layer of striated muscle, **B** (VD only group) shows narrowing of urethral lumen, marked fibrosis with mild periurtethral inflammation, **C** (VD+DM group) shows marked narrowing of urethral lumen, moderate periurtethral inflammation, and severe fibrosis, **D** (VD+ABA group) shows dilated urethral lumen, marked fibrosis with mild periurtethral inflammation, **E** (VD+DM+ABA group) shows narrow urethral lumen (*), mild periurtethral inflammation. H&E, (100x bar 100) urethral lumen (*), inflammation (thin arrow), fibrosis (thick arrow). **(F):** histopathological scoring of urethral changes among different groups by H&E, bars are means±SE, data of urethral lumen diameter was analyzed using one way ANOVA followed by Tukey's test to compare all means. Inflammation scores in urethral wall were statistically analyzed using Kruskal-Wallis One Way ANOVA on Ranks with all pairwise multiple comparison procedures done by Dunn's Method. Different letters mean significant when (P<0.05).

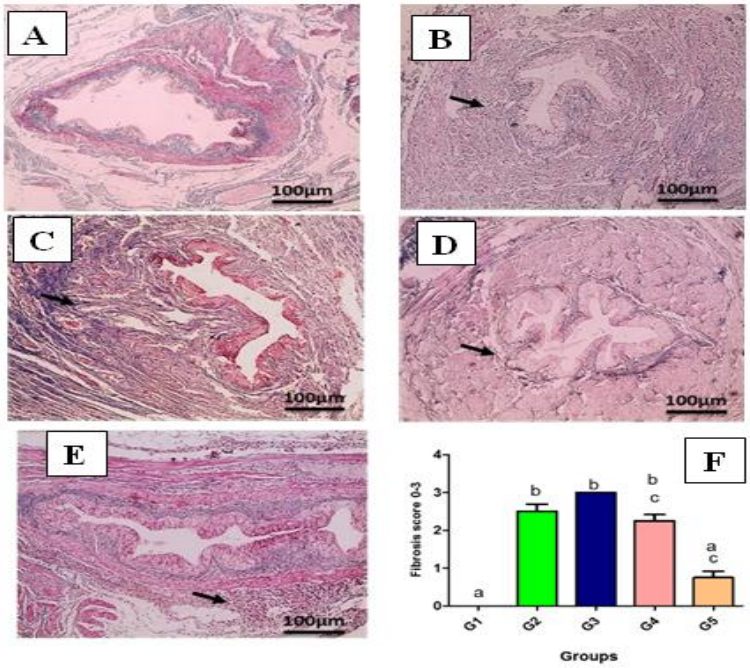

Figure (2): (A-E): Microscopic picture of urethral sections from **A** (control group) shows no fibrosis no excess bluish collagen deposition, **B** (VD only group) shows excess bluish collagen deposition (thin arrow), **C** (VD+DM group) shows marked bluish collagen deposition (thin arrow), **D** (VD+ABA group) shows markedly decreased bluish collagen deposition (thin arrow), **E** (VD+DM+ABA group) shows mild periurtethral bluish collagen deposition (thin arrow). MT, (100x bar 100). **(F):** histopathological scoring of urethral changes among different groups by Masson Trichrome, bars are means±SE. Fibrosis scores in urethral wall were statistically analyzed using Kruskal-Wallis One Way ANOVA on Ranks with all pairwise multiple comparison procedures done by Dunn's Method. Different letters mean significant when (P<0.05).

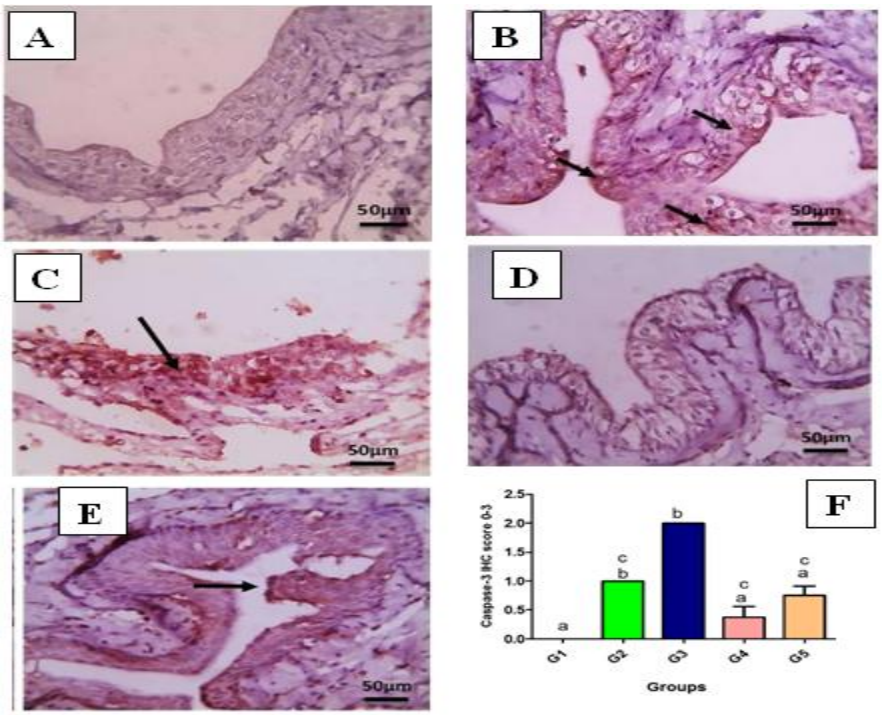

Figure (3): (A-E): Microscopic picture of urethral sections from **A** (control group) shows negative expression for caspase-3, **B** (VD only group) shows positive brown expression for caspase-3 in urothelium (thin arrow), **C** (VD+DM group) shows increased positive brown expression for caspase-3 in urothelium (thin arrow), **D** (VD+ABA group) shows negative expression for caspase-3, **E** (VD+DM+ABA group) shows mild positive brown expression for caspase-3 in urothelium (thin arrow). IHC counterstained with Mayer's hematoxylin, (400x bar 50. **(F):** Morphometric analysis of the mean percentage of caspase-3 immunoreactive area., bars are means±SE. Caspase-3 IHC scores in urethral sections were statistically analyzed using Kruskal-Wallis One Way ANOVA on Ranks with all pairwise multiple comparison procedures done by Dunn's Method. Different letters mean significant when (P<0.05).

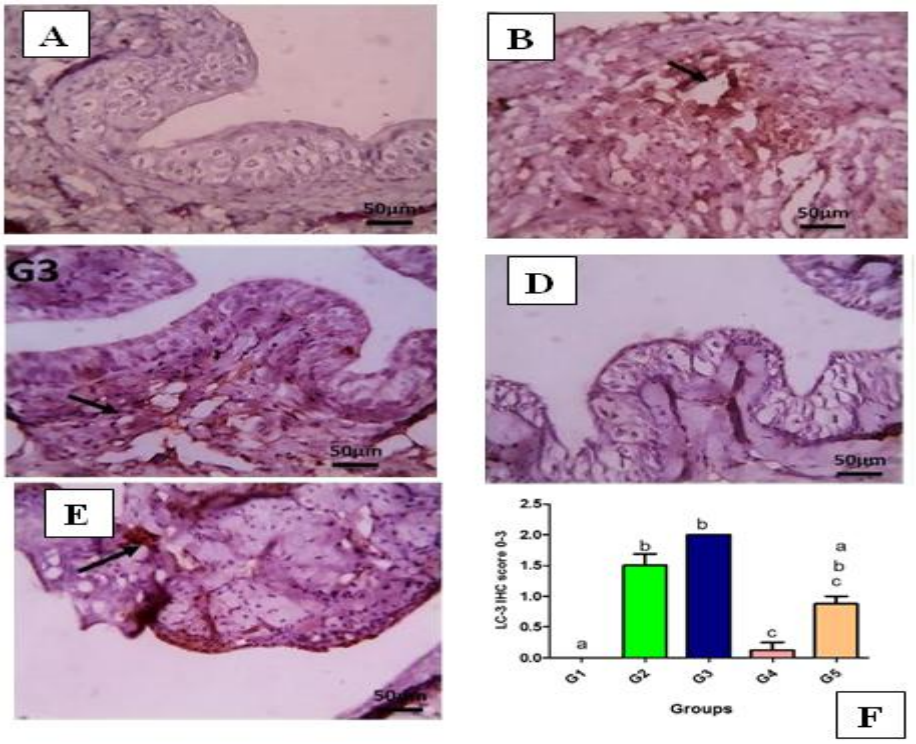

Figure (4): (A-E): Microscopic picture of urethral sections from **A** (control group) shows negative expression for LC-3, **B** (VD only group) shows positive brown expression for LC -3 in lamina propria (thin arrow), **C** (VD+DM group) shows increased positive brown expression for LC-3 in lamina propria (thin arrow), **D** (VD+ABA group) shows negative expression for LC -3, **E** (VD+DM+ABA group) shows mild positive brown expression for LC -3 in lamina propria (thin arrow). IHC counterstained with Mayer's hematoxylin, (400x bar 50). **(F):** Morphometric analysis of the mean percentage of LC-3 immunoreactive area, bars are means±SE. LC-3 IHC scores in urethral sections were statistically analyzed using Kruskal-Wallis One Way ANOVA on Ranks with all pairwise multiple comparison procedures done by Dunn's Method. Different letters mean significant when (P<0.05).

Discussion

In the present study, we investigated the effects of DM and SUI on urethral integrity and function in female Sprague-Dawley rats searching for the potential beneficial role of abscisic acid. Consequently, our study hypothesized that ABA could exert a possible prophylactic and/or therapeutic effect against the combined insults of diabetes and stress-induced urinary incontinence via assessing HbA1c, serum insulin, LDH, urea, creatinine and measurement of abdominal leak point pressure (ALPP) among the different groups. Specimens from urethral origin were obtained for histopathological examination by H&E and Masson's trichrome stains and also immunohistochemical study has been done for expression of Caspase-3 and LC-3.

Our findings showed a substantial increase in HbA1c levels in Group 3 (9.14±0.77) compared to the control group (4.94±0.56), affirming persistent hyperglycemia following STZ administration. This result aligns with previous studies demonstrating STZ's diabetogenic effect due to its selective cytotoxicity to pancreatic β-cells **(22,23)**

We observed that ABA administration in group 5 exhibited non-significant decrease in HbA1c level (8.86 ± 0.64) compared to group 3 (9.14±0.77). A key consideration in interpreting these results is the pharmacokinetic profile and systemic bioavailability of orally administered ABA. The modest decline in HbA1c observed may reflect limited bioavailability or suboptimal dosing for maximum therapeutic effect. Previous studies have noted that while ABA is orally bioavailable, its metabolism and excretion rates vary significantly among species and administration regimens **(13)**. Therefore, higher doses or prolonged administration may produce more pronounced effects, although this requires careful titration to avoid potential toxicity or off- target effects.

In the present study, the significant reduction in serum insulin levels in group 3 (3.12 ± 0.08 ng/mL) compared to the control group (28.75 ± 8.07 ng/mL) confirms the successful establishment of an insulin- deficient diabetic model using streptozotocin (STZ).

The administration of abscisic acid (ABA) in group 5 led to a significant increase in serum insulin (5.56 ± 1.46 ng/mL) compared to Group 3. This finding suggests that ABA possesses β-cell supportive or insulinotropic properties. Although insulin levels did not return to those of non-diabetic groups, the partial recovery indicates a protective or regenerative effect of ABA on pancreatic function. This is consistent with previous studies which reported that ABA can enhance insulin secretion in response to glucose challenges, thereby contributing to better glycemic regulation **(24,25)**. Several studies have proposed that ABA plays a role in glycemic control. It is known to enhance glucose uptake and may stimulate insulin secretion through activation of LANCL2 (lanthionine synthetase C-like protein 2), a receptor involved in metabolic and anti-inflammatory signaling **(13,26)**. Through this receptor, ABA promotes Ca^{2+} influx and cAMP production in β- cells, facilitating insulin exocytosis **(17)**.

Our findings are supported by studies such as **(27)**, who showed that ABA administration improved insulin secretion in rat models of impaired glucose tolerance. Similarly, a previous study **(20)** demonstrated that ABA

supplementation upregulated PPARγ and GLP-1 pathways, further promoting insulin synthesis and pancreatic β-cell preservation.

However, ABA does not directly regenerate β-cells, its anti-inflammatory and antioxidant actions could participate in the preservation of the already existing β-cell mass. Chronic hyperglycemia leads to glucotoxicity, characterized by generation of free radicals, impaired mitochondrial functions, and endoplasmic reticulum (ER) stress, all of which impair β-cell survival **(19)**. ABA has been shown to attenuate oxidative stress markers such as malondialdehyde and enhance levels of antioxidant enzymes such as SOD and glutathione peroxidase **(26)**.

Despite the beneficial effects of ABA, serum insulin levels in group 5 remained significantly lower than in non-diabetic groups (groups 1, 2, and 4). This incomplete recovery highlights the limits of ABA's therapeutic potential in severe β-cell destruction scenarios, such as those induced by high-dose STZ. The residual hypoinsulinemia likely reflects irreversible β-cell loss, emphasizing that while ABA may enhance insulin secretion and reduce glucotoxic stress, it may not be sufficient alone to reverse established β-cell damage. This finding is consistent with research indicating that ABA may be most effective as a preventive or adjunct therapy rather than a curative agent in advanced diabetes **(27)**.

Serum LDH is widely used as a marker of tissue injury and cellular damage **(34)**. The present study revealed that there was a significant increase in LDH levels in group 2 (VD only) compared with control group, also, there was marked increase in LDH in group 3 (VD+DM) compared to group 2, indicating that the combined effects of DM and mechanical injury to the urethra led to substantial tissue damage. This was in accordance with preceding studies which showed that LDH is markedly elevated in diabetic rats **(28)** and also stress-induced urinary incontinence (SUI) causes elevation in oxidative stress markers including LDH **(35)**.

Notably, groups that received ABA (groups 4&5) showed lower LDH levels compared to group 2 (VD only) and group 3 (VD+DM) in significant manner, suggesting that ABA may help to preserve cellular integrity by reducing inflammation and oxidative stress. This observation is consistent with reports indicating that ABA exerts anti-inflammatory and antioxidant effects, thereby protecting tissues from injury **(28)**.

The present study revealed that induction of DM by STZ led to significant increase of serum creatinine (Ser Cr) and urea levels compared to control group. These results reflect compromised renal function, a common complication in diabetic conditions. The kidney is particularly vulnerable to hyperglycemic damage, and elevated levels of these markers often indicate reduced glomerular filtration and renal stress **(36)**. Hyperglycemia triggers multiple pathways that harm the kidneys: increased oxidative stress, formation of advanced glycation end- products, polyol pathway stimulation, and abnormal hemodynamics—all contributing to glomerular hypertrophy, basement membrane thickening, mesangial expansion **(29)**. In our study, group 3 (VD+DM) shows significantly elevated serum urea (58 ± 13 mg/dL) and creatinine (2.07 ± 0.66 mg/dL) compared to both control and VD- only groups

(group 2). The absence of significant renal biomarker changes in Group 2 indicates that mechanical VD alone does not cause notable renal impairment; rather, hyperglycemia drives kidney dysfunction.

Experimental studies align with our findings: single doses of STZ (45 mg/kg) elevate serum urea and

creatinine within 1–4 weeks post‑ injection **(30)**. They also reported up to a 2.66‑ fold increase in creatinine by day 14 post‑ STZ.

It was found that administration of ABA to diabetic rats led to significant decrease of creatinine and urea levels compared to diabetic, suggesting that ABA may have a renoprotective effect. This renoprotective role might be mediated through its anti-inflammatory and antioxidant mechanisms, which have been observed in other models of diabetic complications by decreasing the inflammatory response and reducing oxidative stress, ABA appears to help maintain renal function even in the presence of chronic hyperglycemia and stress-induced injury **(37)**.

Adel et al. (2021) reported that the role of synthetic ABA in improving the renal injury following ischaemic reperfusion injury, with reduction of the tubular necrosis, epithelial sloughing, and cast formation, too. Additionally, they stated that ABA may reduce the oxidative stress marker NOX-4, MDA and increase GSH with a reduction in the apoptotic markers BAX and P53, and an increase in the anti-apoptotic marker Bcl2.

ALPP is a critical parameter of urethral sphincter function, with lower values indicating greater sphincter dysfunction **(39)**. In our study, rats exposed to vaginal dilatation (VD) exhibited a significant decrease in ALPP compared with control group, demonstrating that vaginal dilatation can compromise sphincter function causing urinary incontinence which simulates birth trauma that may occur during vaginal delivery **(40)**. However, group 3 (VD+DM) showed the most severe impairment in ALPP, this may be due to the compounding effects of diabetes via showing periurethral inflammation, significant fibrosis, and atrophy of the muscular layers **(42)** and mechanical trauma by vaginal distention **(45).**

Administration of ABA resulted in significant improvements in ALPP in group 4 (VD+ABA) compared to group 2 (VD only). Moreover, there was a significant increase of ALPP in group 5 (VD+DM+ABA) compared to group 3 (VD+DM). These findings suggest that ABA could exert reparative effects on urethral sphincter and pelvic floor muscles.

The integrity of the urethral structure is essential for maintaining continence **(41).** In control group, the urethral architecture was well-organized with distinct layers, as confirmed by both H&E and Masson trichrome staining. However, in rats with VD, the mechanical insult induced by vaginal distension resulted in narrowing of the urethral lumen. This narrowing was associated with moderate deposition of fibrous tissue in the lamina propria and submucosal layers, which likely represents an early scarring response. This histopathological finding is consistent with previous literature that revealed effect of SUI on urethral layers and lumen **(39)**. In rats with combined VD+DM

(group 3), the histopathological changes were markedly more severe. The urethral lumen was not only narrowed, but there was also extensive periurethral inflammation, significant fibrosis, and atrophy of the muscular layers, a previous study (42) described the effect of diabetes on urethral integrity in female diabetic rats where there is disorganization of muscle layer, signs of cellular atrophy, and inflammatory cell infiltration. Thus, we can conclude the profound effect of combined SUI and diabetes on urethral integrity.

The present study revealed ABA role in ameliorating these histopathological changes. In diabetic rats exposed to VD and received ABA (group 5), the urethral opening was wider, and there was a significant reduction in scarring and inflammation in comparison with rats with VD and DM that not received ABA (group 3). Decreased deposition of fibrous tissue as visualized by Masson trichrome staining, along with the preservation of smooth muscle integrity, indicates that ABA exerts a protective effect on urethral tissue structure. This finding may be attributed to ABA's anti- inflammatory properties, which limit the recruitment and activation of fibroblasts and myofibroblasts that contribute to extracellular matrix deposition and fibrosis **(43)**.

Apoptosis has an essential role in tissue repair. Caspase-3 is a central executioner caspase in the apoptotic pathway, and its expression is often used as a marker of cell death **(44)**.

In our study, immunostaining for caspase-3 revealed significant differences among the groups. Rats underwent to VD only showed a positive reaction against caspase-3 was observed in the urothelium, suggesting that even mechanical stress alone can trigger apoptotic pathways. However, diabetic rats with VD (group 3) exhibited a more significant caspase-3 expression compared to group 2, indicating that the presence of diabetes in combination with SUI exacerbates apoptotic cell death in the urethral tissues. This enhanced apoptosis could contribute to the structural deterioration observed in the histopathological examinations, including the loss of muscle integrity and thinning of the urethral epithelium. Importantly, the administration of ABA caused a notable reduction in caspase-3 expression. There was decreased immunoreactivity for caspase-3 in group 4 (VD+ABA) compared to group 2 (VD only), suggesting that ABA can mitigate the initiation of apoptosis in response to mechanical stress. Even more strikingly, in diabetic rats with VD and received ABA (group 5), caspase-3 expression was notably decreased compared to VD+DM group (group 3). This finding implies that ABA not only reduces the inflammatory and fibrotic responses but also protects against the cellular apoptosis that contributes to tissue degeneration. The anti- apoptotic effect of ABA might be mediated by its ability to modulate intracellular signaling pathways related to oxidative stress and inflammation, thereby promoting cell survival and maintaining tissue integrity **(45)**. This is in agreement with previous literature that shows ABA ameliorates apoptosis and oxidative stress **(33).**

LC3 is widely utilized to assess autophagic activity, particularly the transition from LC3-I to LC3-II, which indicates autophagosome formation. Autophagy is critical for cellular quality control, especially under stress, but dysregulated autophagy can contribute to tissue degeneration **(47)**.

Our findings displayed that there were significant differences in LC3 expression between groups, with implications for both compensatory and maladaptive autophagy in SUI pathogenesis. Rats underwent VD only (group 2) exhibited moderate LC3 upregulation, suggesting that mechanical injury induced autophagic activation. Mild autophagy may initially serve a protective role in removing damaged organelles, however, prolonged or excessive autophagy may lead to cell death and tissue weakening. Similar patterns have been reported in

skeletal muscle injury models, where LC3-II levels increased following mechanical stress **(48)**. Diabetic rats with VD (group 3) demonstrated the most pronounced LC3 expression, suggesting substantial autophagic activity in response to the combined insult of diabetes and mechanical trauma.

In previous study **(49)** suggested that marked increase in LC3 expression may represent a maladaptive autophagic response, potentially leading to autophagy-associated cell death, compounding apoptotic damage. It was found that administration of ABA in rats with VD significantly mitigated LC3 expression, suggesting that ABA modulates autophagy. A study **(50)** reported that ABA may help to maintain autophagic balance by modulating mTOR and AMPK signaling pathways, thereby preserving tissue viability. This regulated autophagy likely contributed to tissue repair and functional recovery after VD. In the present study, diabetic rats underwent VD then received ABA (group 5), showed a significant decrease of LC3 expression compared to groups didn't receive ABA. This suggests that ABA can attenuate hyperglycemia-induced excessive autophagy, restoring it to more adaptive levels.

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Conflict of interest

None.

Reference

1. **Cole JB, Florez JC.** Genetics of diabetes mellitus and diabetes complications. Nat Rev Nephrol. 2020 Jul;16(7):377–90.
2. **Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, et al.** Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol. 2018 May;6(5):361– 9.
3. **Chu PM, Yu CC, Tsai KL, Hsieh PL.** Regulation of oxidative stress by long non-coding RNAs in vascular complications of diabetes. Life. 2022 Feb 12;12(2):274.
4. **Izci Y, Topsever P, Filiz TM, Çınar ND, Uludağ C, Lagro-Janssen T.** The association between diabetes mellitus and urinary incontinence in adult women. Int Urogynecol J. 2009 Aug;20:947–52.
5. **Northwood M.** Understanding the complexity of diabetes and urinary

incontinence in older adults with multiple chronic conditions receiving home care: A mixed-methods study (Doctoral dissertation).

6. **El-Azab AS, Shaaban OM.** Measuring the barriers against seeking consultation for urinary incontinence among Middle Eastern women. BMC Womens Health. 2010 Dec;10:6.
7. **Nazzal Z, Khatib B, Al-Quqa B, Abu-Taha L, Jaradat A.** The prevalence and risk factors of urinary incontinence amongst Palestinian women with type 2 diabetes mellitus: A cross-sectional study. Arab J Urol. 2020 Jan 2;18(1):34–40.
8. **Løwenstein E, Jepsen R, Andersen LL, Laigaard J, Møller LA, Gæde P, et al.** Prevalence of urinary incontinence among women with diabetes in the Lolland-Falster Health Study, Denmark. Neurourol Urodyn. 2021 Mar;40(3):855–67.
9. **Blair Y, Wessells H, Pop-Busui R, Ang L, Sarma AV.** Urologic complications in diabetes. J Diabetes Complications. 2022 Oct 1;36(10):108288.
10. **Powell CR, Gehring V.** Mechanisms of action for diabetic bladder dysfunction—state of the art. Curr Bladder Dysfunct Rep. 2023 Jun;18(2):173–82.
11. **Wittig L, Carlson KV, Andrews JM, Crump RT, Baverstock RJ.** Diabetic bladder dysfunction: a review. Urology. 2019 Jan 1;123:1–6.
12. **Singh A, Roychoudhury A.** Abscisic acid in plants under abiotic stress: Crosstalk with major phytohormones. Plant Cell Rep. 2023 Jun;42(6):961–74.
13. **Magnone M, Sturla L, Guida L, Spinelli S, Begani G, Bruzzone S, et al.** Abscisic acid: A conserved hormone in plants and humans and a promising aid to combat prediabetes and the metabolic syndrome. Nutrients. 2020 Jun 9;12(6):1724.
14. **Bruzzone S, Magnone M, Mannino E, Sociali G, Sturla L, Fresia C, et al.** Abscisic acid stimulates glucagon-like peptide-1 secretion from L-cells and its oral administration increases plasma glucagon-like peptide-1 levels in rats. PLoS One. 2015 Oct 21;10(10):e0140588.
15. **Milborrow BV.** The pathway of biosynthesis of abscisic acid in vascular plants: a review of the present state of knowledge of ABA biosynthesis. Journal of experimental botany. 2001 Jun 1;52(359):1145-64.
16. **Zocchi E, Hontecillas R, Leber A, Einerhand A, Carbo A, Bruzzone S, et al.** Abscisic acid: a novel nutraceutical for glycemic control. Front Nutr. 2017 Jun 13;4:24.
17. **Bruzzone S, Ameri P, Briatore L, Mannino E, Basile G, Andraghetti G, Grozio A, Magnone M, Guida L, Scarfi S, Salis A.** The plant hormone abscisic acid increases in human plasma after hyperglycemia and stimulates glucose consumption by adipocytes and myoblasts. The FASEB Journal. 2012

Mar;26(3):1251-60.

18. **Derosa G, Maffioli P, D'Angelo A, Preti PS, Tenore G, Novellino E.** Abscisic acid treatment in patients with prediabetes. Nutrients. 2020 Sep 24;12(10):2931.
19. **Robertson RP.** Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. Journal of Biological Chemistry. 2004 Oct 8;279(41):42351-4.
20. **Bassaganya-Riera J, Skoneczka J, Kingston DGJ, Krishnan A, Misyak SA, Guri AJ, et al.** Mechanisms of action and medicinal applications of abscisic acid. Curr Med Chem. 2010;17(5):467–78.
21. **Dittmar MS, Fehm NP, Vatankhah B,Horn M.** Ketamine/xylazine anesthesia for radiologic imaging of neurologically impaired rats: dose response, respiratory depression, and management of complications. Comp Med. 2004 Dec 15;54(6):652–5.
22. **Lenzen S.** The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia. 2008 Feb;51(2):216-26.
23. **Szkudelski T.** The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological research. 2001 Jan 1;50(6):537-46.
24. **Bruzzone S, Ameri P, Briatore L, Mannino E, Basile G, Andraghetti G, Grozio A, Magnone M, Guida L, Scarfi S, Salis A.** The plant hormone abscisic acid increases in human plasma after hyperglycemia and stimulates glucose consumption by adipocytes and myoblasts. The FASEB Journal. 2012 Mar;26(3):1251-60.
25. **Zocchi E, Hontecillas R, Leber A, Einerhand A, Carbo A, Bruzzone S, et al.** Abscisic acid: a novel nutraceutical for glycemic control. Front Nutr. 2017 Jun 13;4:24.
26. **Guri AJ, Hontecillas R, Si H, Liu D, Bassaganya-Riera J.** Dietary abscisic acid ameliorates glucose tolerance and obesity- related inflammation in db/db mice fed high-fat diets. Clinical Nutrition. 2007 Feb 1;26(1):107-16.
27. **Booz V.** Abscisic acid: a promising molecule in the treatment of type 2 diabetes mellitus and its complications.
28. **Zhao CC, Xu J, Xie QM, Zhang HY, Fei GH, Wu HM.** Abscisic acid suppresses the activation of NLRP3 inflammasome and oxidative stress in murine allergic airway inflammation Phytother Res. 2021;35(6):3298–309.
29. **Patel, R. P., et al. (2015).** Fructose diet & low- dose STZ on rat nephropathy. Adv Trad Med 15:305–312.
30. **Ahmad Khan, H., & Ola, M. S. (2012).** Renal dysfunction in STZ-diabetic rats. Biomedical Research 23(3):421- 424.
31. **Wszola M, Klak M, Kosowska A, Tymicki G, Berman A, Adamiok-Ostrowska A, et al.** Streptozotocin-induced diabetes in a mouse model (BALB/c) is not an effective model for research on transplantation

procedures in the treatment of type 1 diabetes. Biomedicines. 2021;9(12):1790.

32. **Sakthivel P, Sharma N, Klahn P, Gereke M, Bruder D.** Abscisic acid: a phytohormone and mammalian cytokine as novel pharmacon with potential for future development into clinical applications. Curr Med Chem. 2016;23(15):1549–70.
33. **Bassaganya-Riera J, Skoneczka J, Kingston DGJ, Krishnan A, Misyak SA, Guri AJ, et al.** Mechanisms of action and medicinal applications of abscisic acid. Curr Med Chem. 2010;17(5):467–78.
34. **Drent M, Cobben NA, Henderson RF, Wouters EF, van Dieijen-Visser M.** Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J. 1996;9(8):1736–42.
35. **Chen WH, Jiang YH, Kuo HC.** Urinary oxidative stress biomarkers in the diagnosis of detrusor overactivity in female patients with stress urinary incontinence. Biomedicines. 2023;11(2):357.
36. **Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, Andrade-Sierra J.** Oxidative stress in diabetic nephropathy with early chronic kidney disease. J Diabetes Res. 2016;2016(1):7047238.
37. **Fouda AA, NagehMabroukGabr KRM, Ahmed Mohamed Taha SA, Mokhtar Ahmed Mokhtar Abo-Elfotoh MAM.** Effect of Abscisic Acid on Diabetic Cardiomyopathy in Streptozotocin- Nicotinamide-Induced Diabetic Rats. J Cardiovasc Dis Res. 2021;12(4):1596–612.
38. **Adel M, Rabei MR, Hazem N, Reda Hassan Elsayed H, El-Nablawy MH.** Abscisic Acid Can Protect the Kidney Against Ischemia/Reperfusion Injury Via Antiapoptotic Activity, Downregulation of NOX-4 and Upregulation of Connexin-43. BullEgypt Soc Physiol Sci. 2021;41(4):537–52.
39. **Li CH, Chung MH, Liao CH, Su CC, Lin YK, Liao YM.** Urinary incontinence and sleep quality in older women with type 2 diabetes: a cross-sectional study. Int J Environ Res Public Health. 2022;19(23):15642.
40. **Juliato CRT.** Impact of vaginal delivery on pelvic floor. Rev Bras Ginecol Obstet. 2020;42(2):65–6.
41. **Fırat F, Yalçın K, Erdemir F, Gevrek F.** Evaluation of the Efficacy of Colchicine, Pirfenidone and Prednisolone in Preventing Stricture Due to Inflammation as a Result of Urethral Mucosal Damage in Rats. Evaluation. 2025;12(1):40–8.
42. **Piculo F, Marini G, Barbosa AMP, Damasceno DC, Matheus SMM, Felisbino SL, et al.** Urethral striated muscle and extracellular matrix morphological characteristics among mildly diabetic pregnant rats: translational approach. Int Urogynecol J.

2014;25:403–15.

43. **Jia L, Liu Q, Zheng G, Zhang W, Zhu J, Huang K, et al.** Abscisic Acid Alleviates Cerulein-Induced Acute Pancreatitis and Monosodium Urate-Induced Gouty Arthritis by Inhibiting Nlrp3 Inflammasome Activation. SSRN. Available-from: https://ssrn.com/abstract=5073359
44. **Permatasari HK, Firdaus SDRA, Susanto H, Malek NANN, Sulistomo HW.** Green synthesized Moringa oleifera leaf powder–Silver nanoparticles (MOLP-AgNPs) promotes apoptosis by targeting caspase-3 and phosphorylated-AKT signaling in MCF-7 cells. J Agric Food Res. 2025;19:101640.
45. **Xie L, Huang Y, Zhang T, Li W.** Abscisic acid attenuates apoptosis by modulating oxidative stress and inflammatory signaling pathways in injured tissues. Cell Signal. 2023;105:110522. doi:10.1016/j.cellsig.2023.110522.
46. **Wang L, Zou H, Xiao X, Wu H, Zhu Y, Li J, et al.** Abscisic acid inhibited reactive oxygen species-mediated endoplasmic reticulum stress by regulating the PPAR-γ signaling pathway in ARDS mice. Phytother Res. 2021;35(12):7027–38.
47. **Murrow L, Debnath J. Autophagy** as a stress- response and quality-control mechanism: implications for cell injury and human disease. Annu Rev Pathol. 2013;8(1):105–37.
48. **Sandri M.** Autophagy in health and disease. 3. Involvement of autophagy in muscle atrophy. Am J Physiol Cell Physiol. 2010;298(6):C1291–7.
49. **Lee JH, Park SY, Kim YJ, Choi HJ.** Excessive autophagy contributes to tissue damage: modulation by abscisic acid in a rat model of vaginal distension. Autophagy. 2020;16(5):812–25. doi:10.1080/15548627.2020.1725400.
50. **Lo YH, Chen ML, Zhang Q, Wang X.** Abscisic acid regulates autophagy through mTOR and AMPK signaling to enhance tissue recovery after injury. J Cell Physiol. 2025;240(3):456–68. doi:10.1002/jcp.31025.