



Article

Can *Carica papaya* Serve as an Adjunct to Semaglutide in Mitigating Diabetes-Induced Testicular Injury Through Modulation of Oxidative Stress, Inflammation, Apoptosis, and the miR-34c/miR-155–SIRT1/FOXO1 Axis? An Experimental and Chem-Bio-Informatics Study

Mohamed M. Zeweil ¹, Asmaa F. Khafaga ^{2,*}, Marium M. Shamaa ³, Wafaa Abdelaziz Emam ⁴,
Amena Rezk Mohammed ⁴, Marwa Hassan Sedira ⁴, Safa H. Qahl ⁵, Fatma EL-Zahraa Abd El-Hakam ⁶,
Shih-Min Hsia ^{7,8,9,10,11,*} and Nadia M. Hamdy ¹²

- ¹ Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Egypt; mohamed.zeweil@vetmed.dmu.edu.eg
 - ² Department of Pathology, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Saudi Arabia
 - ³ Biochemistry Department, Clinical and Biological Sciences Division, College of Pharmacy, Arab Academy for Science Technology and Maritime Transport, Alexandria 1029, Egypt; marium.muhammed@aast.edu
 - ⁴ Medical Biochemistry Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo 11884, Egypt; wafaaabdelaziz.medg@azhar.edu.eg (W.A.E.); amenarezk.medg@azhar.edu.eg (A.R.M.); mrwahhassan.medg@azhar.edu.eg (M.H.S.)
 - ⁵ Department of Biological Sciences, College of Science, University of Jeddah, P.O. Box 80327, Jeddah 21589, Saudi Arabia; shqahal@uj.edu.sa
 - ⁶ Pharmacology Department, Faculty of Medicine for Girls, Al-Azhar University, Nasr City, Cairo 11884, Egypt; fatmaabdelhakem.medg@azhar.edu.eg
 - ⁷ School of Nutrition and Health Sciences, College of Nutrition, Taipei Medical University, Taipei 110301, Taiwan
 - ⁸ School of Food Safety, Taipei Medical University, Taipei 110301, Taiwan
 - ⁹ Nutrition Research Center, Taipei Medical University Hospital, Taipei 110301, Taiwan
 - ¹⁰ Graduate Institute of Metabolism and Obesity Sciences, College of Nutrition, Taipei Medical University, Taipei 110301, Taiwan
 - ¹¹ TMU Research Center for Digestive Medicine, Taipei Medical University, Taipei 110301, Taiwan
 - ¹² Biochemistry and Molecular Biology Department, Faculty of Pharmacy, Ain Shams University, Abassia, Cairo 11566, Egypt; nadia_hamdy@pharma.asu.edu.eg
- * Correspondence: akhfajah@kfu.edu.sa (A.F.K.); bryanhsia@tmu.edu.tw (S.-M.H.)

Abstract

Diabetes mellitus (DM) induces significant endocrine disruption and oxidative stress (OS) within the testes, resulting in impaired spermatogenesis, increased sperm abnormalities, and compromised reproductive function. This study aimed to evaluate the combined protective effects of Semaglutide (SEM) combined with *Carica papaya* (papaya) juice against type 2 diabetes-induced testicular damage in rats. Forty adult male albino rats were divided into four experimental groups: a control group, a Streptozotocin (STZ)-induced diabetic group, a diabetic group treated with SEM (0.3 mg/kg), and a diabetic group treated with SEM (0.3 mg/kg) in combination with 10% papaya juice, administered for eight weeks. Statistically significant superiority over SEM alone was observed for selected endpoints; the findings primarily support the potential of papaya as a dose-sparing adjunct rather than demonstrating uniformly enhanced efficacy. They significantly improved systemic metabolic parameters, as evidenced by reduced fasting blood glucose (FBG) and glycated hemoglobin (HbA_{1c}) levels and restoration of the lipid profile. Importantly, it also attenuated diabetes-induced testicular injury, as demonstrated by improved reproductive hormone levels, enhanced sperm parameters, restoration of antioxidant defenses, modulation of inflammatory and apoptotic signaling, and marked histopathological recovery of



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seminiferous tubular architecture. Antioxidant markers revealed a notable reduction in malondialdehyde (MDA) and cytochrome P450 2E1 (CYP2E1), along with significant increases in reduced glutathione, catalase (CAT), and superoxide dismutase (SOD). Furthermore, a marked modulation of key pro-inflammatory and pro-apoptotic mediators was observed, including forkhead box protein O1 (FOXO1), microRNA-155 (miR-155), tumor necrosis factor- α (TNF- α), nuclear factor kappa B cell subunit 1 (NF- κ B1), interleukin-6 (IL-6), caspase-3 (CASP3), and BCL2-Associated X Apoptosis Regulator (Bax), while a significant upregulation of sirtuin-1 (SIRT1), microRNA-34c (miR-34c), and B-cell lymphoma-2 (Bcl-2) was also detected. Histopathological assessments confirmed the restoration of normal testicular architecture in the treated groups. These findings indicate that the combination strategy may have the potential to achieve dose savings while maintaining efficacy comparable to the standard-dose SEM, through the enhancement of the antioxidant defenses, modulation of inflammation, and apoptosis, specifically via the modulation of the miR-34c/miR-155 and SIRT1/FOXO1 signaling.

Keywords: Semaglutide (SEM); testicular damage; papaya (*Carica papaya*); apoptosis; miR-155; miR-34c/SIRT1/FOXO1; in silico analysis; chem-bioinformatics

1. Introduction

Type 2 diabetes mellitus (T2DM) accounts for over 90% of all diabetes cases and is marked by chronic hyperglycemia due to impaired insulin secretion and/or action [1–4]. In addition to its systemic metabolic burden, T2DM significantly impairs male reproductive health, reducing sperm quality, inducing testicular damage, and promoting DNA fragmentation [5]. These effects are largely driven by oxidative stress (OS) triggered by hyperglycemia, leading to lipid peroxidation, inflammation, and apoptosis within the testicular tissue [6].

Emerging evidence highlights the role of microRNAs (miRNAs; miR) in modulating several pathological processes/diseases such as cancer and diabetes [7–10]. For instance, miR-155 is upregulated during inflammation and suppresses sirtuin 1 (SIRT1), a key deacetylase involved in cellular stress resistance and metabolic regulation [11,12]. On the other hand, miR-34c, abundantly expressed in testicular tissue, is essential for spermatogenesis, and its downregulation contributes to infertility [13,14]. SIRT1 interacts closely with FOXO1, a transcription factor vital for cell survival, OS defense, and spermatogonial maintenance [15]. The SIRT1/FOXO1 axis thus plays a central role in protecting testicular integrity under diabetic conditions.

Despite advances in glycemic management, current therapies remain limited in preventing or reversing diabetic testicular injury [16,17]. Therefore, identifying potential agents that can modulate both the metabolic and the inflammatory pathways is of growing interest. Glucagon-like peptide-1 (GLP-1) receptor agonists have demonstrated anti-inflammatory and antioxidant properties beyond their glucose-lowering effects [18]. SEM, a GLP-1 receptor agonist, regulates metabolic parameters and shows promise in mitigating diabetes-associated tissue damage [19,20]. Likewise, *Carica papaya*, rich in the antioxidant vitamins A, C, and E and bioactive enzymes like papain, offers anti-inflammatory and cytoprotective effects, with potential applications in chronic metabolic conditions [21].

To date, limited evidence is available regarding the combined therapeutic impact of SEM and papaya on diabetic testicular damage; therefore, the current study was designed to evaluate papaya as an adjunct to SEM rather than as monotherapy. Thus, independent effects of SEM and papaya (SEM + papaya) in attenuating testicular injury in STZ-induced

diabetic rats were examined in the current study. It is noteworthy to mention that the rationale for employing the add-on therapy in the present study was to investigate whether co-administration of papaya juice could enhance the protective efficacy of SEM (0.3 mg/kg) and thereby support a potential dose-sparing therapeutic strategy while maintaining biological effectiveness against diabetes-induced testicular injury. This approach is particularly relevant given the well-recognized dose-dependent adverse effects of GLP-1 receptor agonists, including gastrointestinal intolerance and systemic complications reported at higher doses. Moreover, we will focus on the metabolic and reproductive parameters, OS markers, the expression of miRs 155 and 34c as well as apoptotic signals, namely SIRT1, FOXO1, TNF- α , NF- κ B1, IL-6, CASP3, Bax, and Bcl-2, to explore the mechanistic and therapeutic implications of this novel potential combination.

2. Results

Throughout the experiment, rats in all groups ($n = 10$ per group), except the control group, exhibited symptoms such as increased body weight, polyphagia, polyuria, and polydipsia. Neither the control group nor the other treated groups displayed any apparent symptoms or mortality.

2.1. Effect of SEM + Papaya on Diabetic Profile of STZ-T2DT Rats

As shown in Table 1, serum levels of FBG and HbA_{1c} were significantly higher in the STZ-treated group compared to the control group. In contrast, treatment with SEM (0.3 mg/kg) alone or in combination with papaya in STZ-intoxicated rats led to a marked reduction ($p < 0.001$) in these levels compared to diabetic rats. (The blood glucose change curves during the experiment are included in Supplementary file S1.)

Table 1. Protective effects of SEM + papaya on FBG, HbA_{1c}, and lipids profile in STZ-induced diabetic rats.

Parameter (Unit)	FBG (mg/dL)	HbA _{1c} (%)	TAG (mg/dL)	VLDL (mg/dL)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Group							
Control	91.333 $\pm$ 1.097	4.330 $\pm$ 0.296	137.90 $\pm$ 2.27	27.58 $\pm$ 0.454	124.533 $\pm$ 3.192	51.92 $\pm$ 4.88	45.033 $\pm$ 2.89
STZ	262.166 $\pm$ 8.147 ^a	8.99 $\pm$ 0.482 ^a	280.70 $\pm$ 2.08 ^a	56.14 $\pm$ 0.42 ^a	222.066 $\pm$ 1.835 ^a	137.693 $\pm$ 1.68 ^a	28.233 $\pm$ 1.77 ^a
STZ + SEM 0.3	142.200 $\pm$ 6.335 ^{a,b}	5.423 $\pm$ 0.304 ^{a,b}	171.50 $\pm$ 5.99 ^{a,b}	34.30 $\pm$ 1.19 ^{a,b}	161.2 $\pm$ 1.802 ^{a,b}	92.9 $\pm$ 0.87 ^{a,b}	34.00 $\pm$ 0.78 ^{a,b}
STZ + SEM 0.3 + Papaya	146.366 $\pm$ 5.365 ^{a,b}	5.543 $\pm$ 0.361 ^{a,b}	158.43 $\pm$ 1.41 ^{a,b,c}	31.686 $\pm$ 0.28 ^{a,b,c}	147.866 $\pm$ 1.8 ^{a,b,c}	81.38 $\pm$ 0.83 ^{a,b,c}	34.80 $\pm$ 1.31 ^{a,b}

Data are presented as the mean $\pm$ SE of the mean ($n = 10$ rats/group). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for post hoc comparisons.

^a Statistically significantly different from the control group; ^b statistically significantly different from the STZ group; ^c statistically significantly different from the STZ + SEM (0.3 mg/kg) group. Statistically significant differences are set at $p < 0.05$. (STZ: Streptozotocin; SEM: Semaglutide; FBG: fasting blood glucose; HbA_{1c}: glycated hemoglobin; TAG: triacylglycerol; VLDL: very-low-density lipoprotein; TC: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol.).

2.2. Effect of SEM + Papaya on Diabetic and Lipid Profile of STZ-T2DT Rats

Serum levels of TAG, VLDL, TC, and LDL were significantly higher in the STZ-treated group compared to the control group, as shown in Table 1. In contrast, treatment with SEM (0.3 mg/kg) or SEM + papaya in STZ-intoxicated rats led to a marked reduction ($p < 0.001$) in these levels, compared to the STZ-treated group. However, the levels of HDL were significantly decreased in STZ-treated rats compared to control rats and significantly increased ($p < 0.0001$) in groups treated with SEM, either alone or with papaya, compared to STZ-treated rats.

2.3. Effect of SEM + Papaya on Fertility Profile of STZ-T2DT Rats

As illustrated in Table 2, similar trends ($p < 0.001$) were observed for serum levels of testosterone (total, free, and bioavailable), LH, and 17 β -estradiol. Notably, a significant difference was evident between the groups receiving SEM (0.3 mg/kg) alone and those

treated with the SEM + papaya combination. The STZ-treated group exhibited a marked increase in estradiol levels alongside a significant reduction in testosterone (total, free, and bioavailable) and LH levels compared to the control group. Conversely, administration of SEM + papaya to STZ-induced rats led to a substantial decrease in estradiol concentrations ($p < 0.0001$) and a significant increase in testosterone (total, free, and bioavailable) and LH levels ($p < 0.0001$) compared to both the STZ-treated and SEM-only groups.

Table 2. Protective effects of SEM + papaya on fertility biomarkers (SHBG (nmol/L), total/free/bioavailable testosterone (pg/mL), LH (mIU/mL), and estradiol (pg/mL)) in STZ-induced diabetic rats.

Group/Parameter (Unit)	SHBG (nmol/L)	T.testo (pg/mL)	F.testo (pg/mL)	B.testo (pg/mL)	LH (mIU/mL)	E2 (pg/mL)
Control	14.458 ± 0.584	3.4 ± 0.173	0.096 ± 0.005	2.017 ± 0.168	4.23 ± 0.171	24.5 ± 0.734
STZ	29.275 ± 1.969 ^a	1.67 ± 0.185 ^a	0.033 ± 0.004 ^a	0.69 ± 0.088 ^a	2.78 ± 0.075 ^a	40.53 ± 2.165 ^a
STZ + SEM 0.3	18.842 ± 0.909 ^{a,b}	2.4 ± 0.153 ^b	0.06 ± 0.001 ^{a,b}	1.27 ± 0.107 ^{a,b}	3.34 ± 0.041 ^a	34.32 ± 1.263 ^{a,b}
STZ + SEM 0.3 + Papaya	15.415 ± 0.447 ^{a,b,c}	2.43 ± 0.088 ^b	0.067 ± 0.002 ^{a,b}	1.41 ± 0.072 ^{a,b}	3.88 ± 0.086 ^a	31.66 ± 1.164 ^{a,b,c}

Data are presented as the mean ± SE of the mean (n = 10 rats/group). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for post hoc comparisons. ^a Statistically significantly different from the control group; ^b statistically significantly different from the STZ group; ^c statistically significantly different from the STZ + SEM (0.3 mg/kg) group. Statistically significant differences are set at $p < 0.05$. (STZ: Streptozotocin; SEM: Semaglutide; SHBG: sex-hormone-binding globulin; T.testo: total testosterone; F.testo: free testosterone; B.testo: bioavailable testosterone; LH: luteinizing hormone; E2: estradiol (17 β -estradiol)).

2.4. Effect of SEM + Papaya on Various Sperm Parameters of STZ-T2DT Rats

Figure 1 illustrates the detrimental impact of STZ-induced diabetes on various sperm parameters and the potential ameliorative effects of SEM and papaya co-treatment. Panel A shows a significant reduction ($p < 0.0001$) in sperm count in the STZ group compared to controls, while SEM (0.3 mg/kg) and SEM + papaya treatments were partially associated with restoration of sperm concentration, although they were not identical to the control. In Panel B, immotile sperm percentage significantly increased ($p < 0.0001$) in diabetic rats, whereas SEM (0.3 mg/kg) and especially the SEM + papaya combination significantly reduced ($p < 0.0001$) this parameter compared to diabetic rats, suggesting improved sperm motility. Panel C highlights a significant decline ($p < 0.0001$) in total sperm motility due to STZ, with treatments demonstrating a significant recovery, particularly with SEM + papaya compared to diabetic rats. Finally, Panel D indicates a significant ($p < 0.0001$) rise in abnormal sperm morphology in the diabetic group, which was notably ameliorated in the treatment groups, though not fully restored to control levels.

2.5. Effect of SEM + Papaya on Oxidants and Anti-Oxidants of STZ-T2DT Rats

As presented in Table 3, the STZ-induced diabetic group demonstrated a significant reduction ($p < 0.0001$) in the enzymatic activities of CAT and SOD, as well as in the non-enzymatic antioxidant GSH. In contrast, levels of Cyp2e1 and MDA were significantly elevated ($p < 0.0001$) compared to the control group. Administration of SEM combined with papaya to STZ-intoxicated rats was markedly associated with restoration of the antioxidant status, as evidenced by significantly increased GSH levels and enhanced CAT and SOD activities, alongside a notable reduction in CYP2E1 and MDA levels ($p < 0.0001$) relative to the untreated STZ group. Although these parameters did not differ significantly from those in the SEM-only group, they remained substantially different from the control values ($p < 0.0001$).

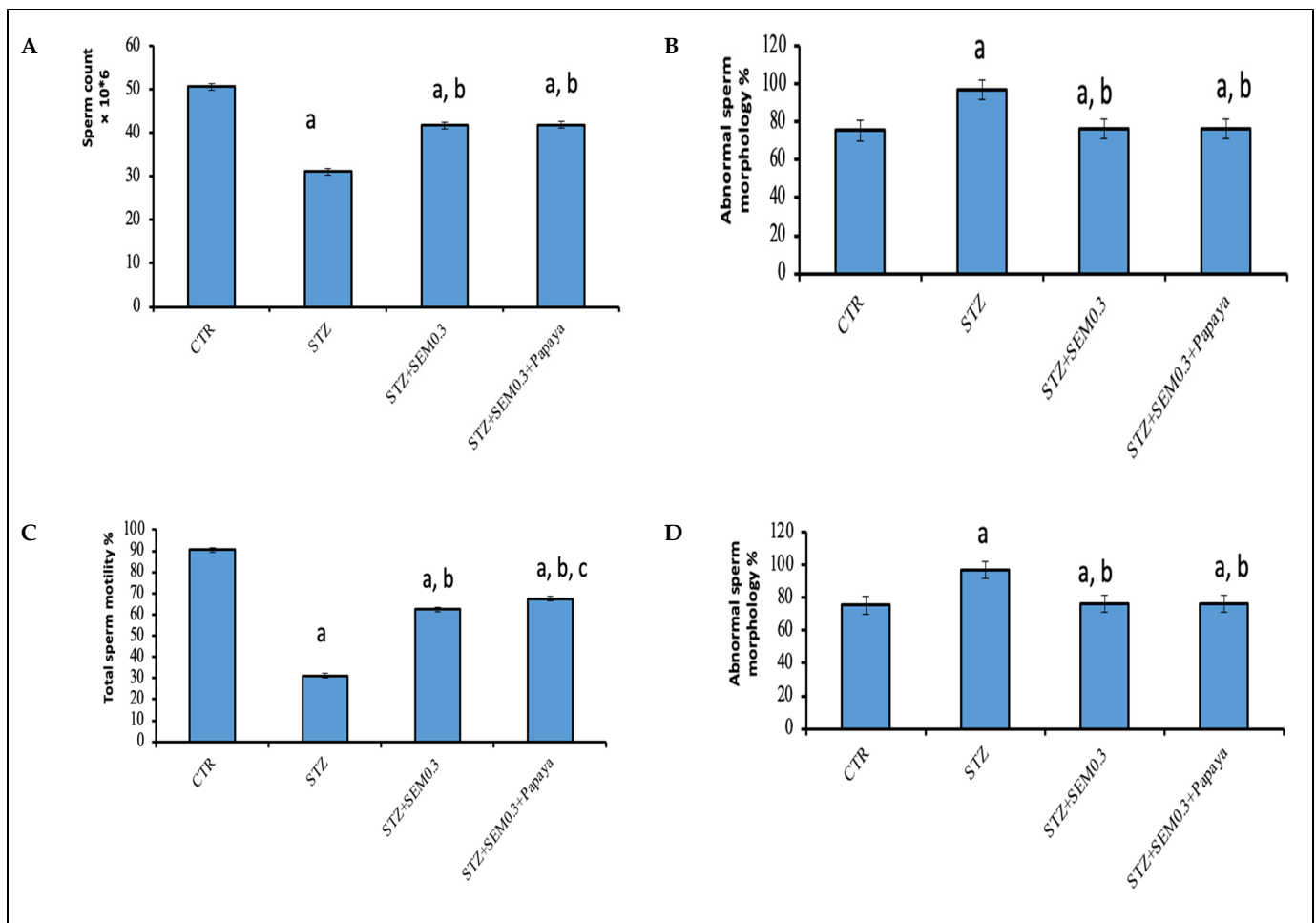


Figure 1. Effects of STZ-induced diabetes and subsequent treatments on sperm parameters (%). (A) Sperm count ($\times 10^6$); (B) immotile sperm (%); (C) total sperm motility (progressive + non-progressive) (%); and (D) abnormal sperm morphology (%), across different groups: control (CTR), diabetic (STZ), diabetic treated with SEM (STZ + SEM (0.3 mg/kg)), and diabetic treated with SEM and papaya (STZ + SEM (0.3 mg/kg) + papaya). ^a statistically significantly different from the control group; ^b statistically significantly different from the STZ group; ^c statistically significantly different from the STZ + SEM (0.3 mg/kg) group. Statistically significant differences are set at $p < 0.05$.

Table 3. Impact of treatment with SEM (0.3 mg/kg) or SEM (0.3 mg/kg) + papaya on oxidants (MDA and Cyp2e1) and antioxidants (GSH, CAT, and SOD) of STZ-intoxicated rats compared to control rats.

Group/Parameter (Unit)	MDA (nmol/g. Tissue)	Cyp2e1 (ng/mL)	GSH (nmol/g. Protein)	CAT (U/mg. Protein)	SOD (U/mg. Protein)
Control	23.09 $\pm$ 1.2	62.38 $\pm$ 1.8	14.647 $\pm$ 1.42	56.58 $\pm$ 0.518	17.35 $\pm$ 1.23
STZ	47.29 $\pm$ 1.28 ^a	128.857 $\pm$ 1.21 ^a	6.41 $\pm$ 1.28 ^a	20.62 $\pm$ 1.13 ^a	8.27 $\pm$ 0.58 ^b
STZ + SEM 0.3	31.23 $\pm$ 1.06 ^{a,b}	88.36 $\pm$ 1.13 ^{a,b}	11.5 $\pm$ 1.184 ^{a,b}	42.74 $\pm$ 1.7 ^{a,b}	11.83 $\pm$ 0.96 ^{a,b}
STZ + SEM 0.3 + Papaya	29.84 $\pm$ 1.59 ^{a,b}	75.69 $\pm$ 1.45 ^{a,b,c}	12.28 $\pm$ 1 ^{a,b}	41.76 $\pm$ 1.8 ^{a,b}	13.97 $\pm$ 0.56 ^{a,b}

Data are presented as the mean $\pm$ SE of the mean ($n = 10$ rats/group). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for post hoc comparisons. ^a Statistically significantly different from the control group; ^b statistically significantly different from the STZ group; ^c statistically significantly different from the STZ + SEM (0.3 mg/kg) group. Statistically significant differences are set at $p < 0.05$. (STZ: Streptozotocin; SEM: Semaglutide; MDA: malondialdehyde; Cyp2e1: cytochrome p450 2E1; GSH: reduced glutathione; CAT: catalase; SOD: superoxide dismutase.).

2.6. Effect of SEM + Papaya on SIRT1/FOXO1 of STZ-T2DT Rats

As illustrated in Figure 2A, FOXO1 mRNA expression was markedly elevated ($p < 0.0001$), whereas SIRT1 expression (Figure 2B) was significantly reduced ($p < 0.0001$)

in the STZ-treated rats compared to the control group. Administration of the SEM and papaya combination effectively reversed this pattern, resulting in a significant modulation of FOXO1 and upregulation of SIRT1 ($p < 0.0001$) relative to the STZ group. However, these changes remained significantly different ($p < 0.0001$) when compared with the SEM-only group.

2.7. Modulation of Pro-Inflammatory mRNA Expression (TNF- α , NF- κ B1, and IL-6) by SEM + Papaya in STZ-Induced Diabetic Rats

As depicted in Figure 2C–E, the STZ group exhibited a marked elevation ($p < 0.0001$) in the mRNA expression of TNF- α , NF- κ B1, and IL-6, respectively, relative to control animals, indicating a heightened inflammatory response. However, treatment with either SEM (0.3 mg/kg) alone or in combination with papaya resulted in a significant suppression ($p < 0.0001$) of these inflammatory markers compared to the STZ group, highlighting the anti-inflammatory potential of the intervention.

2.8. Regulation of miR-155 and miR-34c by SEM + Papaya in STZ-Induced Diabetic Rats

Figure 2F demonstrates that miR-155 was significantly upregulated, whereas miR-34c was downregulated ($p < 0.0001$) in the STZ group compared to controls. Notably, administration of SEM alone or in combination with papaya significantly reduced miR-155 levels and elevated miR-34c expression ($p < 0.0001$) relative to the untreated diabetic group, suggesting a reversal of fibrosis-associated molecular alterations.

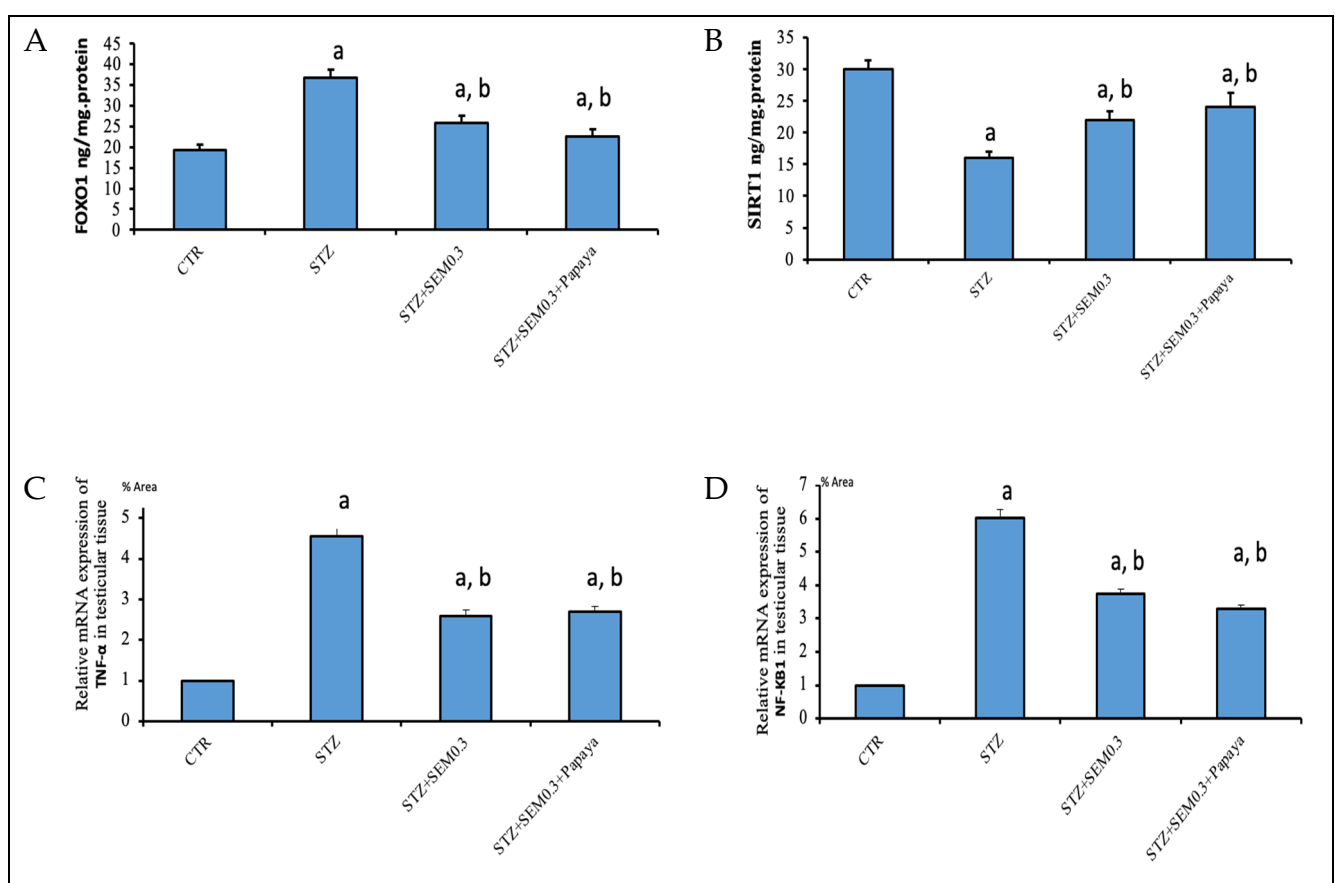


Figure 2. Cont.

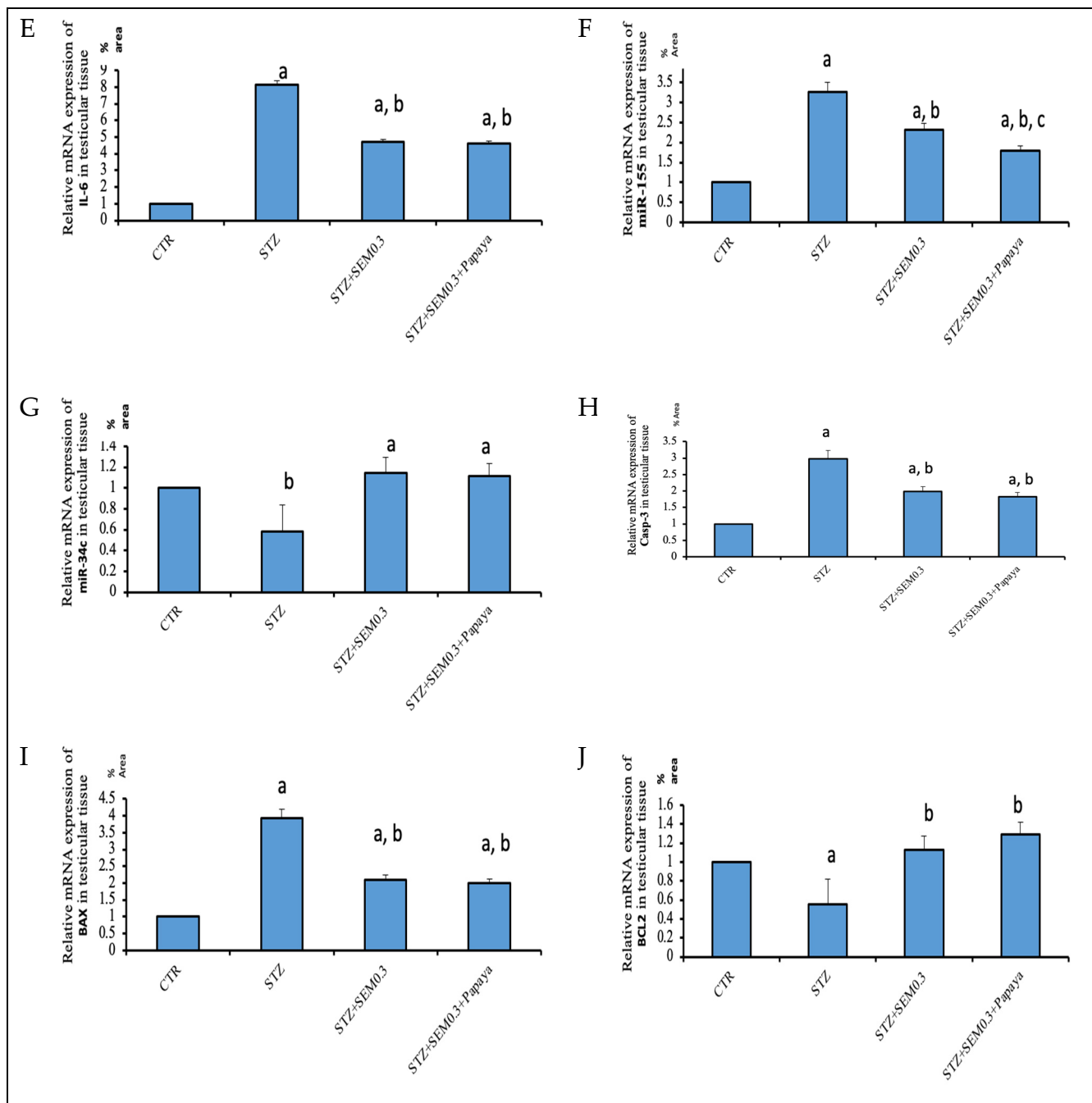


Figure 2. Effects of STZ-induced diabetes and subsequent treatments on (A) FOXO1 (ng/mg protein), (B) SIRT1 (ng/mg protein), (C) TNF- α , (D) NF- κ B1, (E) IL-6, (F) miR-155, (G) miR-34c, (H) CASP3, (I) BAX, and (J) BCL-2 across different groups: control (CTR), diabetic (STZ), diabetic treated with SEM (STZ + SEM (0.3 mg/kg)), and diabetic treated with SEM and papaya (STZ + SEM (0.3 mg/kg) + papaya). Values are represented as mean $\pm$ SE of the mean ($n = 10$ /group). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for post hoc comparisons. ^a statistically significantly different from the control group; ^b statistically significantly different from the STZ group; ^c statistically significantly different from the STZ + SEM (0.3 mg/kg) group. Statistically significant differences are set at $p < 0.05$.

2.9. Anti-Apoptotic Effect of SEM + Papaya on mRNA Expression of CASP3, Bax, and Bcl-2 in STZ-Induced Diabetic Rats

As illustrated in Figure 2H–J, the STZ-treated rats exhibited significant overexpression of the pro-apoptotic genes CASP3 and Bax, along with suppressed expression of the anti-

apoptotic gene Bcl-2 ($p < 0.0001$), compared to the control group. Conversely, administration of SEM (0.3 mg/kg) or SEM + papaya led to a notable downregulation of CASP3 and Bax and an upregulation of Bcl-2 ($p < 0.0001$) versus the STZ group, demonstrating a protective anti-apoptotic response.

2.10. Histopathological Assessment

Figure 3 illustrates the normal histological architecture of the testicular interstitium in control groups (Figure 3A), including well-defined Leydig cells, an intact basement membrane, and a regular germinal epithelium lining the seminiferous tubules. In contrast, testicular sections from STZ-treated rats displayed severe degenerative changes. These included degeneration of the seminiferous tubules, vacuolar disruption of the germinal epithelium, and interstitial edema evidenced by pale eosinophilic material (Figure 3B). Additionally, marked congestion of interstitial blood vessels (Figure 3C) and irregular, buckled basement membranes (Figure 3D) were observed. Conversely, testicular tissues from diabetic rats treated with SEM alone (Figure 3E) or in combination with papaya extract (Figure 3F) exhibited substantial histological restoration. Only mild vacuolization of the germinal epithelium was noted, indicating a notable protective or therapeutic effect of SEM and papaya extract.

Table 4 (mean $\pm$ st error of the mean Johnsen scores per group for Figure 3 images) presents the semi-quantitative evaluation of spermatogenesis based on the Johnsen scoring system and indicates severe impairment (reduction; severe tubular degeneration, loss of spermatogenic layers, and vacuolization) in the STZ group (scores $\approx$ 3–6) spermatogenesis, whereas treatment with SEM partially restored spermatogenesis (significantly improved seminiferous tubular integrity) (scores $\approx$ 7–8). Notably, the SEM + papaya combination produced near-normal seminiferous tubular architecture (a greater restoration of spermatogenic activity; preserved basement membrane and spermatogenic sequence) (scores $\approx$ 8–9), approaching control levels (scores $\approx$ 9–10) (complete spermatogenesis, dense germinal epithelium, abundant spermatozoa).

Table 4. Semi-quantitative evaluation of spermatogenesis using the Johnsen scoring system in the studied groups.

Group	Johnsen Score
Control	9.62 $\pm$ 0.21
STZ	3.84 $\pm$ 0.27 ^a
STZ + SEM 0.3	7.48 $\pm$ 0.34 ^{a,b}
STZ + SEM 0.3 + Papaya	8.91 $\pm$ 0.29 ^{a,b,c}

Data are presented as mean $\pm$ SE of the mean ($n = 5$ sections/group). Statistical analysis was performed using one-way ANOVA followed by Duncan's post hoc test. ^a Significantly different from control group ($p < 0.05$); ^b significantly different from STZ group ($p < 0.05$); ^c significantly different from STZ + SEM (0.3 mg/kg) group ($p < 0.05$).

2.11. Cheminformatics Studies of the Repurposed SEM

2.11.1. In Silico Measurement of Physicochemical Properties and Tox21

The physicochemical and pharmacokinetic properties of SEM were predicted using the ADMETlab 3.0 online platform (<https://admetlab3.scbdd.com/server/evaluation> (accessed on 18 October 2025)). The evaluated parameters included the logarithm of the n-octanol/water distribution coefficient at pH 7.4 (logD7.4), acid–base dissociation constant (pKa), plasma protein binding (PPB), mitochondrial membrane potential toxicity (SR-MMP), aromatase activity (NR-Aromatase), and aryl hydrocarbon receptor activity (NR-AhR). As summarized in Table 5, SEM exhibited a logD7.4 of -0.6 and a pKa range of 0.715–6.864, indicating low lipophilicity and poor blood–brain barrier permeability, suggesting a low

likelihood of central nervous system exposure and associated neurotoxicity. Toxicity predictions based on the Tox21 Data Challenge framework, developed under the Toxicology in the 21st Century (Tox21) program by the NIH and FDA, are presented in Table 5.

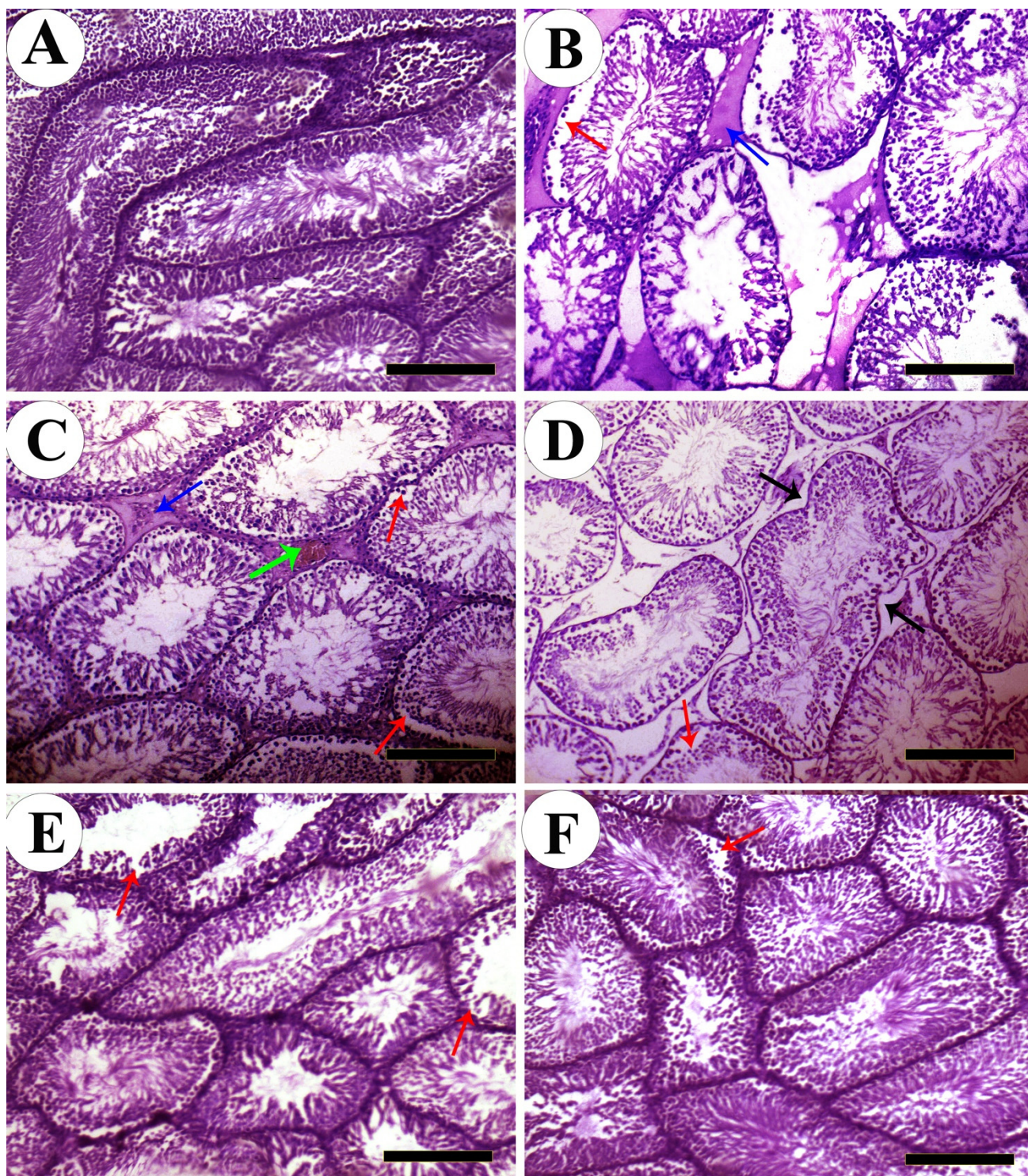
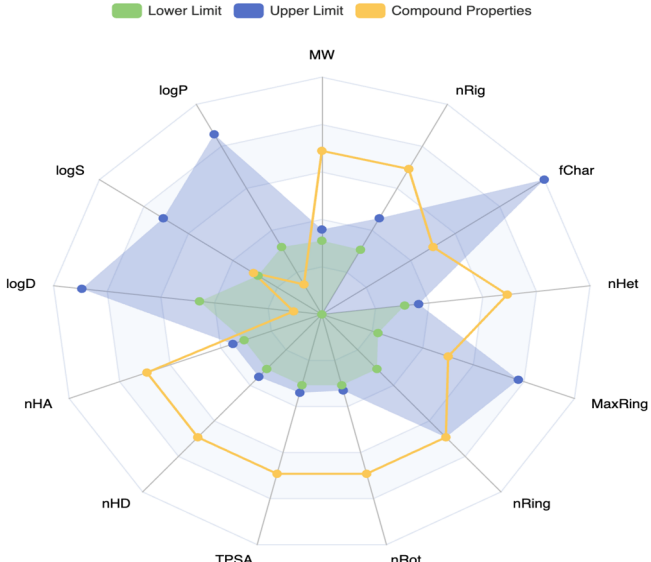


Figure 3. Representative photomicrographs of testicular tissue sections histopathological assessment (H&E). (A) The control group shows normal histological features, including intact seminiferous tubules lined with well-organized germinal epithelium, clearly defined Leydig cells in the interstitial space, and a regular basement membrane. (B–D) STZ-treated rats exhibit marked testicular damage characterized by degeneration of seminiferous tubules, vacuolar degeneration of the germinal epithelium (red arrows),

interstitial edema (blue arrows), congestion of interstitial blood vessels (green arrow), and irregular and buckled basement membranes (black arrows). (E) SEM (0.3 mg/kg) and (F) SEM + papaya-treated diabetic groups demonstrate notable histological improvement, with preserved seminiferous tubule architecture and only mild vacuolization of the germinal epithelium (red arrows). Scale bar = 100 μ m. Five samples/group. Smears were examined under a Leica DM 3000 light microscope (by Leica Microsystems, Wetzlar, Germany) set to $\times$ 400 magnifications, utilizing a $\times$ 40 objective lens for precise observation.

Table 5. Semaglutide predicted physicochemical properties and TOX21.

Parameter	Sem	Comment (Radar View)
Mwt. g/mol	4111.12	
LogD7.4	−0.6	
pKa (acid–base)	0.715–6.864	
PPB	31.9%	
SR-MMP	---	Inactive
NR-Aromatase	---	Inactive
NR-AhR	---	Inactive
BBB Penetration	---	Inactive

SEM C187H291N45O59 per SMILES. <https://pubchem.ncbi.nlm.nih.gov/compound/56843331> (accessed on 18 October 2025). CC[C@H](C)[C@@H](C(=O)N[C@@H](C)C(=O)N[C@@H](CC1=CNC2=CC=CC=C21)C(=O)N[C@@H](CC(C)C)C(=O)N[C@@H](C(C)C)C(=O)N[C@@H](CCCNC(=N)N)C(=O)NCC(=O)N[C@@H](CCCNC(=N)N)C(=O)NCC(=O)O)NC(=O)[C@H](CC3=CC=CC=C3)NC(=O)[C@H](CCC(=O)O)NC(=O)[C@H](CCCCNC(=O)COCCOCCNC(=O)COCCOCCNC(=O)CC[C@H](C(=O)O)NC(=O)CCCCCCCCCCCCCCCCC(=O)O)NC(=O)[C@H](C)NC(=O)[C@H](C)NC(=O)[C@H](CCC(=O)N)NC(=O)CNC(=O)[C@H](CCC(=O)O)NC(=O)[C@H](CC(C)C)NC(=O)[C@H](CC4=CC=C(C=C4)O)NC(=O)[C@H](CO)NC(=O)[C@H](CO)NC(=O)[C@H](C(C)C)NC(=O)[C@H](CC(=O)O)NC(=O)[C@H](CO)NC(=O)[C@H](C[C@H](C)O)NC(=O)[C@H](CC5=CC=CC=C5)NC(=O)[C@H](C[C@H](C)O)NC(=O)CNC(=O)[C@H](CCC(=O)O)NC(=O)C(C)C)NC(=O)[C@H](CC6=CN=CN6)N.
<https://admetlab3.scbdd.com/server/evaluationCal> (accessed on 18 October 2025) (logD7.4: the logarithm of the n-octanol/water distribution coefficient at pH= 7.4; pKa: acid–base dissociation constant; PPB: plasma protein binding; SR-MMP: mitochondrial membrane potential; NR-AhR: nuclear receptor-aryl hydrocarbon receptor; BBB: blood–brain barrier).

CTD analysis (<https://ctdbase.org/detail.go?type=chem&acc=C000591245> (accessed on 18 October 2025); CID:56843331, SID:497612847) revealed that genes interacting with SEM were significantly enriched in pathways related to TNF signaling, apoptosis, NOD-like receptor signaling (SIRT1), NF- κ B signaling, insulin resistance, and IL and cytokine signaling, and immune system regulation was identified as a key regulatory component within these interconnected pathways.

2.11.2. Bioinformatics Databases Analysis

Immune cell infiltration functional enrichment analysis (Figure 4) Accessed 18 October 2025.

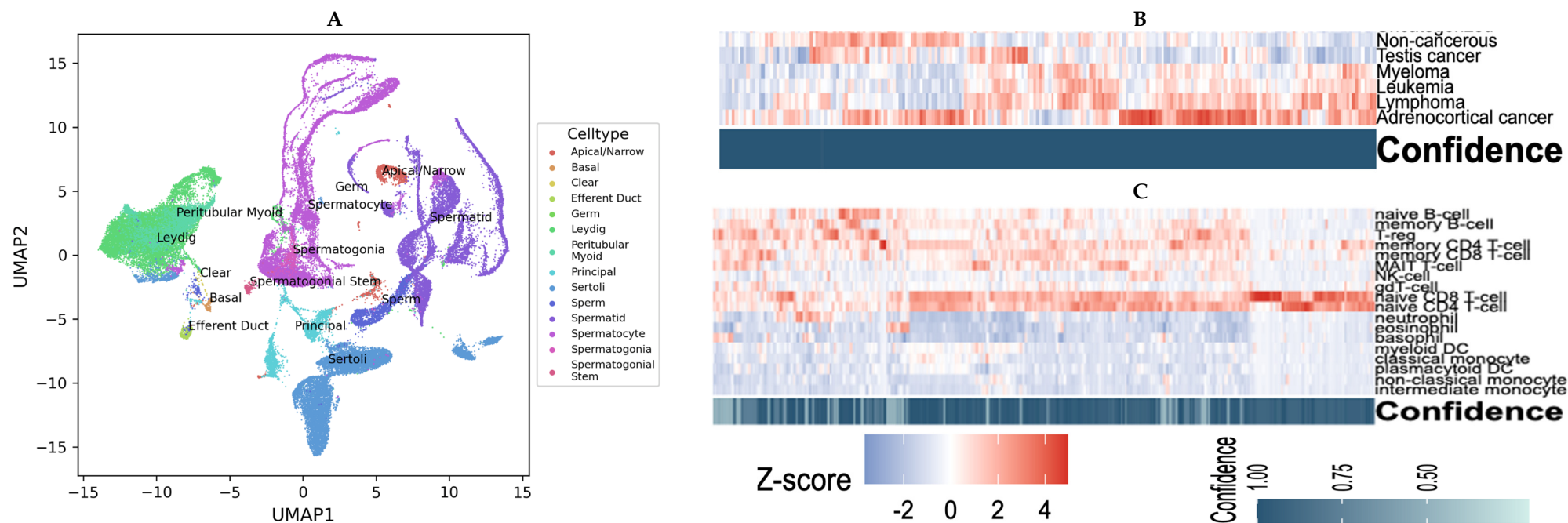


Figure 4. Cont.

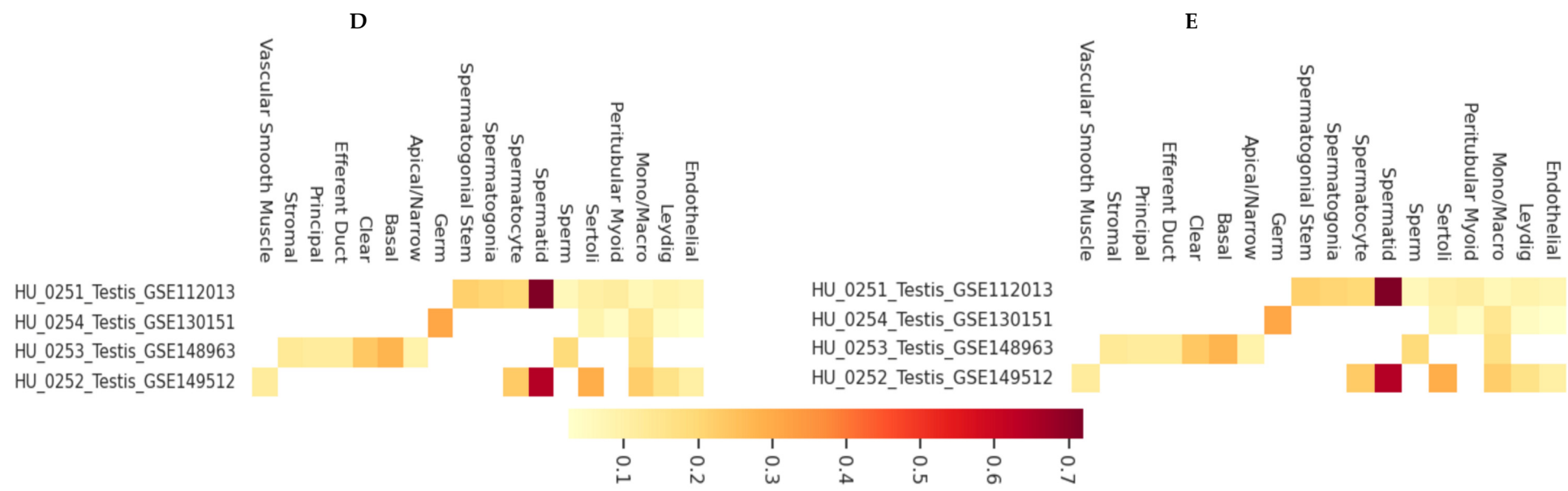


Figure 4. Testis tissue specific annotation details (A) and (B,C) SIRT1 and FOXO1 genes’ relative expression heatmaps from 0.0 to 1.0 (relative expression legend is from white (0.0) to red (0.5) to dark red (1.0)) and confidence from 0.0 to 1.00 (confidence legend is from white (0.0) to light blue (0.50) to blue (0.75) and finally to dark blue (1.00 confidence)). N.B.: SIRT1 is part of cluster 52, addressing 220 genes, with confidence 1 (dark blue), FOXO1 is part of cluster 43, addressing 433 genes, with confidence 0.99 (dark blue). <https://www.proteinatlas.org/humanproteome/cell+line/expression+cluster#cluster52> (accessed on 18 October 2025) and <https://www.proteinatlas.org/humanproteome/single+cell/immune+cell/expression+cluster#cluster43> (accessed on 18 October 2025), respectively. Gene relative expression heatmap in the testis tissue for (D) SIRT1 and (E) FoxO1, retrieved from http://husch.comp-genomics.org/#/info_tissue/Testis (accessed on 18 October 2025).

Testis-specific single-cell expression profiles of SIRT1 and FOXO1 were analyzed using HUSCH (http://husch.comp-genomics.org/#/info_tissue/Testis (accessed on 18 October 2025)) and validated using HPA (<https://www.proteinatlas.org/> (accessed on 18 October 2025)). Figure 4A presents the testis tissue annotation, Figure 4B,C show the immune cell cluster distribution of SIRT1 and FOXO1, respectively (<https://www.proteinatlas.org/humanproteome/cell+line/expression+cluster#cluster52> (accessed on 18 October 2025); <https://www.proteinatlas.org/humanproteome/single+cell/immune+cell/expression+cluster#cluster43> (accessed on 18 October 2025)), and Figure 4D,E illustrate their relative expression heatmaps in testicular tissue, respectively.

Protein–Protein Interaction (PPI) and Functional Network Analysis (Figure 5)

Using GeneMANIA, STRING v12.0, and STITCH v5.0, Figure 5A depicts the predicted genes' (miR-155, miR-34c, CASP3, SIRT1, FOXO1, BAX, BCL2, TNF- α , NF- κ B1, and IL-6) interaction network, whereas Figure 5B presents the integrated protein interaction network and functional associations, including pathways, co-expression, co-localization, and protein-domain similarity, highlighting the principal molecular interactions underlying testicular injury (accessed 16 October 2025).

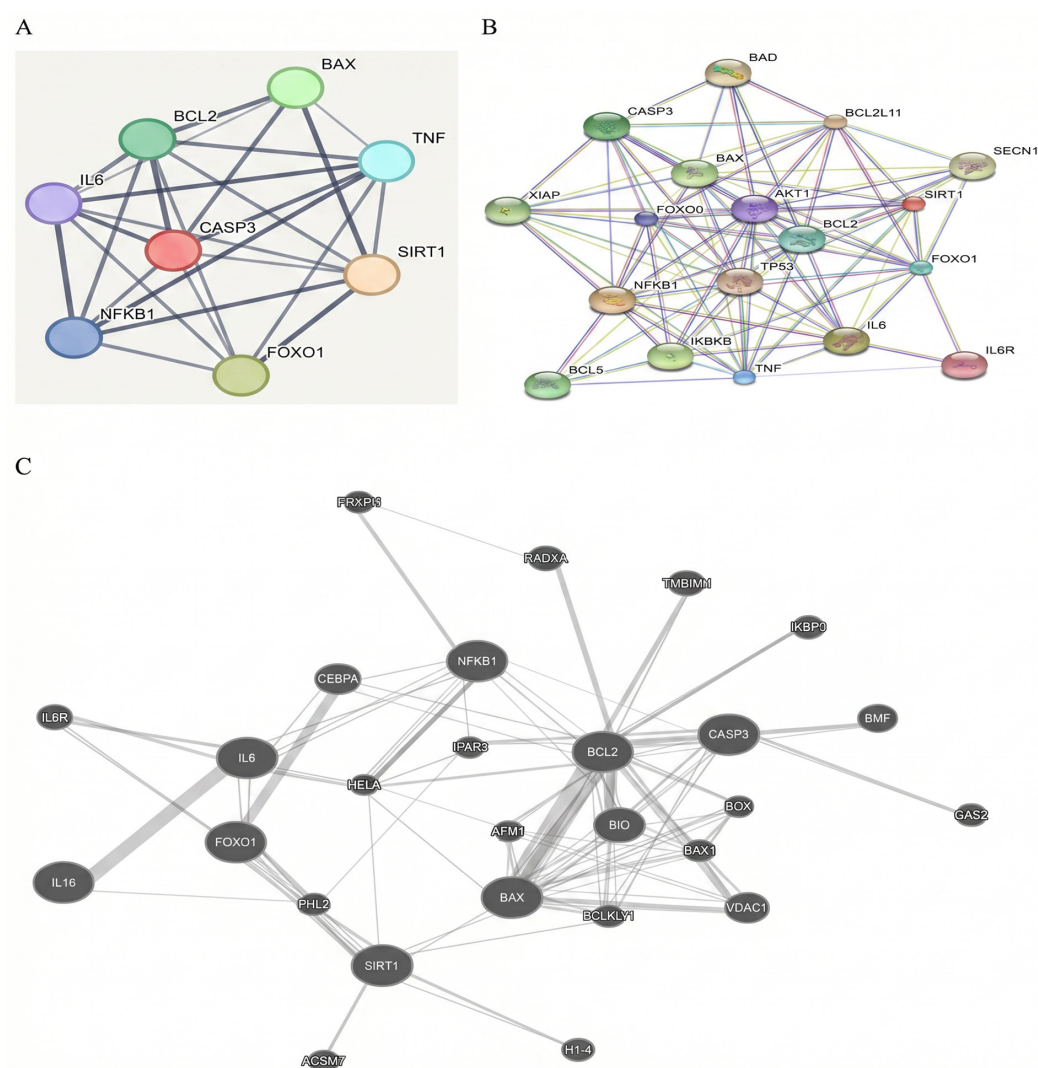


Figure 5. Protein–protein interaction (PPI) network. (A) STRING, (B) STITCH, and (C) GeneMANIA (<https://genemania.org/search/homo-sapiens/miR-155/miR-34c/Casp3/Bax/Bcl2/Tnf-%CE%B1/NF-%CE%BAB1/IL-6/sirt1/FOXO1> (accessed on 18 October 2025)) presentation of predicted functional partners' network (accessed 18 October 2025).

3. Discussion

Among the various deleterious complications of T2DM [22] is testicular injury, which often leads to compromised male infertility [23].

The present study evaluated the effects associated with adding *C. papaya* juice to SEM treatment in rats with STZ-induced diabetic testicular injury. Both SEM-containing regimens improved several metabolic, reproductive, oxidative stress, inflammatory, apoptosis-related, and histopathological outcomes compared with the untreated diabetic group. However, the combination treatment was not consistently superior to SEM alone across the measured endpoints. In particular, FBG, HbA1c, testosterone, and several oxidative-stress markers did not differ significantly between the two SEM-containing groups. Therefore, the findings should not be interpreted as demonstrating a general enhancement of SEM efficacy by papaya. Rather, they indicate that the combination produced broadly comparable effects to SEM alone, with additional improvement limited to selected outcomes.

Diabetic testicular damage is frequently associated with structural alterations in testicular tissue, disrupted male hormone profiles, reduced sperm motility, and elevated rates of sperm abnormalities [24]. In line with previous studies, our T2DT group exhibited a marked reduction in serum testosterone and LH levels [22,25,26]. Histopathological evaluation of testicular tissues revealed pronounced seminiferous tubule degeneration, congestion of interstitial blood vessels, vacuolar degeneration of the germinal epithelium, irregular and buckled basement membrane of seminiferous tubules, interstitial edema that was represented by faint eosinophilic material, and a notable increase in apoptotic germ cells. These findings are consistent with earlier observations [27,28].

Importantly, OS emerges as a central pathogenic mechanism driving testicular atrophy, germ cell loss, hormonal disruption, and increased sperm abnormalities [29,30]. Taken together, these pathological outcomes underscore the multifaceted impact of T2DM on male reproductive health. The groups treated with SEM and papaya extract exhibited a significant enhancement in sperm count and motility [31,32]. The observed reduction in sperm quality in the STZ group is likely due to OS exerted on spermatogenic cells [33]. Notably, spermatozoa are particularly susceptible to oxidative damage because of their high content of polyunsaturated fatty acids (PUFAs) in the plasma membrane and low concentrations of antioxidant enzymes in the cytoplasm, leading to infertility via cytotoxic radical generation that harms both spermatogenic cells and testicular tissue [34]. This aligns with the elevated testicular ROS and MDA levels and depleted antioxidant defenses (GSH, catalase, and SOD) [35,36].

In the present study, the SEM + papaya group showed partial restoration of antioxidant status and sperm parameters relative to the untreated diabetic group. However, several oxidative stress and reproductive outcomes did not differ significantly between the SEM and SEM + papaya groups. Therefore, these findings do not establish that papaya significantly enhanced the antioxidant or testicular protective effects of SEM. Erectile function was not evaluated in the present study, and no conclusions regarding erectile dysfunction can be drawn.

Hyperglycemia further amplifies this damage by promoting excessive ROS production in the testes, sperm, and epididymis, triggering apoptosis of both germ and somatic cells [37,38]. ROS also impairs sperm viability and motility by damaging DNA, oxidizing proteins, and inducing lipid peroxidation [25]. The altered transcript levels of Bcl-2, Bax, and CASP3 observed in the diabetic group were consistent with changes in apoptosis-related gene expression [37,38]. However, apoptosis was not directly confirmed because cleaved caspase-3, caspase activity, and TUNEL staining were not assessed.

CYP2E1 represents a superfamily of membrane-bound hemoprotein isozymes whose primary role lies in the metabolism of xenobiotics and clearance of potentially harmful

metabolites. Notably, SEM has been reported to alleviate diabetes-induced testicular damage by downregulating CYP2E1 expression within the testicular tissue [39]. It is noteworthy to mention that we specifically measured CYP2E1 isoform because it is a major source of ROS and is significantly upregulated in diabetic testis. CYP2E1 is constitutively expressed in Leydig cells, and its induction by hyperglycemia contributes directly to testicular oxidative damage through increased H₂O₂ and lipid peroxidation. Therefore, CYP2E1 serves as a relevant mechanistic biomarker for OS in diabetic testicular injury, unlike other hepatic-dominant CYP450 isoforms.

ROS can influence several pro-inflammatory transcriptional programs [40]; therefore, the transcriptional upregulation of IL-6, NF- κ B1, and TNF- α observed in the current study aligns with previous findings of STZ-induced testicular inflammation through NF- κ B1 activation and proinflammatory cytokine expression [41]. Inflammation is widely recognized as a central contributor to hyperglycemia and its complications [42,43]. Therefore, reducing inflammatory cytokine expression is a viable strategy to alleviate diabetes-induced testicular damage [23,44]. IL-6, NF- κ B1, and TNF- α inhibit Leydig cell function and suppress LH-induced testosterone synthesis [45,46]. These cytokines also interfere with sustentacular cell activity and spermatogenic enzyme expression, thereby contributing to testicular dysfunction. Notably, natural products have been shown to downregulate IL-6, NF- κ B, and TNF- α expression in testicular injury models [47–49], which is consistent with our findings. In the present study, STZ exposure increased the testicular transcript levels of TNF- α , NF- κ B1, and IL-6, whereas both SEM-containing treatments reduced the expression of these genes relative to the untreated diabetic group. These findings demonstrate treatment-associated alterations in inflammatory-gene expression. However, because only NF- κ B1 transcript abundance was measured, the present results do not establish activation or inhibition of NF- κ B signaling. Confirmation of NF- κ B activity would require assessment of NF- κ B protein abundance, phosphorylation, nuclear translocation, or transcriptional activity. Therefore, the present findings should be limited to changes in NF- κ B1 gene expression rather than interpreted as direct regulation of NF- κ B signaling.

One of the central features of diabetic testicular dysfunction is interstitial fibrosis, an irreversible pathological process [50]. This disrupts the testicular microenvironment, impairs testosterone production and spermatogenesis, and ultimately leads to male infertility and sexual dysfunction. Consequently, limiting testicular fibrosis is a strategic focus for mitigating diabetic testicular injury [51]. A pivotal player in this context is NF- κ B1, a multifunctional cytokine involved in cell division regulation [52]. It is secreted primarily by Leydig and sustentacular cells within the testis, operating through both paracrine and endocrine pathways [53]. Mounting evidence indicates that overexpression of such cytokines promotes sperm cell apoptosis and activates testicular fibroblasts, two key events contributing to reproductive failure [54]. Apoptosis is considered an important contributor to diabetic testicular injury [55–57]. Previous studies have linked an increased Bax/Bcl-2 ratio and CASP3 activation to germ-cell apoptosis in diabetic testes [58,59]. This imbalance between the pro-apoptotic Bax and the anti-apoptotic Bcl-2 shifts the cell fate toward death. Hyperglycemia exacerbates this by triggering the release of cytochrome C from the mitochondria into the cytoplasm, subsequently activating CASP3 and promoting DNA fragmentation, hallmarks of apoptosis in spermatogenic cells [60–62]. Interestingly, treatment with natural products appears to reverse this molecular imbalance. In diabetic rats, elevated Bcl-2 levels alongside reduced Bax and CASP3 expression have been observed following such interventions [63,64]. In the present study, the observed changes in Bax, Bcl-2, and CASP3 transcript levels were consistent with alterations in apoptosis-related gene expression. Nevertheless, these expression-level changes do not demonstrate inhibition of apoptosis or direct modulation of apoptotic signaling. Functional confirmation using

cleaved caspase-3, caspase activity, TUNEL staining, or other apoptosis-specific assays is required.

The papaya effects are likely mediated by its rich content of vitamins and phytochemicals [65,66], notable presence of saponins, flavonoids, cardiac glycosides, and deoxy-sugar. Moderate levels of anthraquinones and alkaloids were also detected [22], while phenols and tannins were absent, underscoring the distinct bioactive profile of this natural extract. Papaya fruit is rich in bioflavonoids associated with anti-toxic, anti-inflammatory, and anticancer pharmacological activities [67,68].

From an endocrine perspective, the interstitial cells of the testis play a central role in androgen production [69]. Testosterone, a key male hormone, is essential for spermatogenesis and the development of secondary sexual characteristics [61,69,70]. The production and regulation of testosterone are governed by LH, while follicle-stimulating hormone (FSH) primarily regulates spermatogenesis [71–73]. In diabetic conditions, a decline in testosterone is often accompanied by damage to both the seminiferous tubules and testicular interstitium [74].

Our results corroborate those of [75], who reported a significant decline in serum testosterone levels due to STZ administration, which adversely affects both spermatogenesis and the structure of seminiferous tubules. However, administration of SEM and papaya effectively reversed the histological damage and was associated with restoration of testosterone synthesis, as evidenced by an increase in serum testosterone levels and H&E staining. These findings indicate that both SEM-containing regimens were associated with partial recovery of testicular structure and reproductive hormone profiles. However, because a diabetic papaya-only group was not included, the specific contribution of papaya cannot be determined. In our study, STZ-treated rats exhibited significantly reduced levels of total, free, and bioavailable testosterone, alongside a decrease in serum LH levels, and an increase in estradiol (E2) and SHBG. These findings align with those of [76]. Our study demonstrated that treatment with SEM and papaya resulted in an increased total, free, and bioavailable testosterone and serum LH levels, coupled with decreased SHBG and E2 levels. These hormonal changes were accompanied by improved testicular histology. Nevertheless, the present design does not establish that papaya independently protected testicular tissue or directly stimulated testosterone synthesis in Leydig cells. Studies have shown that the regulation of the LH–Leydig cell axis is altered when Leydig cells are isolated or when the germinal epithelium is disrupted, leading to changes in circulating hormone levels [77–79]. This phenomenon may explain the hormonal variations observed in our study.

miR-155 and miR-34c have been implicated in inflammation, spermatogenesis, and diabetes-related complications [80–86]. In the present study, STZ exposure was associated with increased miR-155 expression, decreased miR-34c expression, reduced SIRT1 protein abundance, and increased FOXO1 protein abundance. Both SEM-containing regimens shifted these measures toward the control values. These findings indicate an association among treatment, miRNA expression, and SIRT1/FOXO1 protein abundance. Direct target engagement was not examined, and FOXO1 phosphorylation, acetylation, nuclear localization, and transcriptional activity were not assessed. Therefore, the proposed regulatory axis should be regarded as a hypothesis-generating mechanistic model that requires further functional validation. Sirt1, a class III protein deacetylase, regulates DNA activity and influences ROS production and FoxO [87,88]. Our results indicate that OS triggered by free radicals modulates Sirt1 mRNA expression [89,90], thus impacting spermatogenesis. Our findings suggest that miR-155/miR-34c/Sirt1/FoxO1 play a role in testicular apoptosis induced by T2DM. Specifically, we observed a decrease in Sirt1 mRNA expression associated with lower miR-34c expression in the testes of STZ-treated rats. These results highlight the

critical involvement of OS and apoptosis in testicular damage induced by STZ, mediated through the miRs/Sirt1/FoxO1 signaling axis.

SIRT1 gene pathway members are TNF (<http://signalink.org/node/Q96EB6> (accessed on 18 October 2025)); post-translational modifiers are androgen receptors (ARs), FOXO1, and tissue protein 53 (TP53); translational regulators are FOXO, TP53, TNF, and ILs. FOXO1 gene pathway members are AKT1 and PPARG (<http://signalink.org/node/Q12778> (accessed on 18 October 2025)); post-translational modifiers are BCL2, TP53, ARs, and SIRT1. Further insulin interaction transcriptional regulators are ARs, IL6, and TNF. This result is evident when interacting via the STRING version 12.0 database and STITCH 5.0 databases and BioGRID ^{5.0} (<https://thebiogrid.org/116983/summary/homo-sapiens/sirt1.html> (accessed on 18 October 2025) and <https://thebiogrid.org/108597/summary/homo-sapiens/foxo1.html> (accessed on 18 October 2025)) and CTD (<https://ctdbase.org/detail.go?type=chem&acc=C000591245> (accessed on 18 October 2025)), and if using UCSC (<https://genome-euro.ucsc.edu/index.html> (accessed on 18 October 2025)), the tool used is gene interactions for identifying gene interactions and pathways from curated databases and text-mining (https://genome-euro.ucsc.edu/cgi-bin/hgGeneGraph?gene=&1=OK&supportLevel=text&geneCount=10&geneAnnot=drugbank&1=OK&hgsid=408051079_fDI4SX9E8tFhfWguWSGRZnbDCCUZ&geneCount=10 (accessed on 18 October 2025) and <https://genome-euro.ucsc.edu/cgi-bin/hgGeneGraph?gene=FOXO1&1=OK&supportLevel=text&geneCount=15&1=OK&geneCount=25&geneAnnot=drugbank> (accessed on 18 October 2025)).

Motivated by these encouraging outcomes, our ongoing research is expanding the scope per these findings to investigate their therapeutic efficacy across other diabetes-related complications, including those affecting the brain [22]. Moreover, diabetic complications of more natural compounds affecting the kidneys, liver, and additional vital organs [91–94] are being tested, examining the effects on genetic/epigenetics and more signaling pathways. Based on the current and projected benefits, we advocate for integrating natural products such as *C. papaya* with chemotherapy [95] where drug-related adverse effects present significant barriers to health-care.

Limitations: Direct functional evidence of apoptosis, NF- κ B activation, and FOXO1 activity was not obtained. Future studies should employ a factorial design incorporating papaya-only, SEM-only, and combination groups at multiple doses, together with functional pathway assays.

4. Materials and Methods

4.1. Chemicals, Drugs, Reagents, and Kits

Streptozotocin (STZ) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Semaglutide (SEM) was purchased from Novo Nordisk (Bagsværd, Denmark) subcutaneous injector, administered subcutaneously. The cDNA synthesis kit and qRT-PCR master mix and RevertAid™ Synthesis Kit were purchased from Qiagen (Hilden, Germany). Forward and reverse primers specific to miR-155, miR-34c, TNF- α , NF- κ B1, IL-6, CASP3, Bax, and Bcl-2 were procured from Sigma-Aldrich (St. Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) kits were purchased from MyBioSource (San Diego, CA, USA) for the following analytes: rat cytochrome P450 2E1 (CYP2E1; Cat. No. MBS2023931), sirtuin 1 (SIRT1; Cat. No. MBS2600246), forkhead box protein O1 (FOXO1; Cat. No. MBS749342), sex-hormone-binding globulin (SHBG; Cat. No. MBS902439), total/free/bioavailable testosterone (Cat. No. MBS702057), luteinizing hormone (LH; Cat. No. MBS764675), and 17 β -estradiol (Cat. No. MBS263466). Kits used for assessing OS biomarkers were obtained from Bio-Diagnostics Co. (Cairo, Egypt), including reduced glutathione (GSH; Cat. No. GR2511), catalase (Cat. No. CAT2517), superoxide dismutase (SOD; Cat. No. SD2521),

and malondialdehyde (MDA; Cat. No. MD2529). Biochemical assay kits for FBG (Cat. No. 10260), HbA1c (Cat. No. 10770), triacylglycerol (TAG; Cat. No. 10724), total cholesterol (TC) (Chol; Cat. No. 10028), high-density lipoprotein cholesterol (HDL-C) (HDLc; Cat. No. 10284), and low-density lipoprotein cholesterol (LDL-C) (LDLc; Cat. No. 10294) were purchased from Human Diagnostics Worldwide (Wiesbaden, Germany). While very-low-density lipoprotein (VLDL) was calculated as TAG/5. Unless otherwise specified, all other chemicals utilized in this study were also acquired from Sigma-Aldrich. Additionally, all reagents used were of the highest analytical grade and purity to ensure the accuracy and reliability of the experimental results.

4.2. Preparation of Papaya Fruit Pulp Juice

Fresh papaya fruits (*Carica papaya*) were obtained from local markets in Damanhour City, Egypt. The fruits were thoroughly washed and peeled, and the seeds were discarded to preserve the integrity of the edible pulp. The cleaned pulp was homogenized using a blender in a 1:1 ratio (500 g of pulp per 500 mL) with sugar-free distilled water. The resulting mixture was then diluted to prepare a 10% (*w/v*) papaya juice solution. The juice was stored in sterile containers at -20°C until further use [22]. The phenolic metabolites were separated, identified, and quantified using a PerkinElmer PE200 HPLC system equipped with an autosampler, column oven, and binary pump, according to the method described in [96]. The analysis was performed with an injection volume of 20 μL and a flow rate of 0.7 mL/min under ESI negative ionization mode with a dwell time of 50 ms, employing MRM transitions. Standard working solutions were prepared by diluting the stock standards in the mobile phase. Compound concentrations were calculated from calibration curves generated using chromatographic peak areas. All solvents used were of HPLC grade, filtered, and degassed prior to analysis. The phytochemical and main phenolic metabolites of the papaya pulp are total phenol mg expressed as gallic acid equivalents/g of extract with a conc. mean, from three replicate measurements, of 66.33, and total flavonoids mg expressed as quercetin equivalents/g of extract around 125, and 2,2 Diphenyl-1-picrylhydrazyl (%) 58, as per the previous study by Zeweil, et al. [22].

Sample size: In accordance with the 3Rs principles, OCED guidelines, and ACUC recommendations, for animal rights and use, the minimum number of animals necessary for valid experimental outcomes was employed. The sample size was determined via power analysis using the “resource equation” method [97] and based on our previous work [22]. All procedures were conducted in full compliance with the US National Research Council’s Guide for the Care and Use of Laboratory Animals (2011) and the ARRIVE guidelines. Strict measures were adopted throughout the study to ensure the highest standards of animal welfare, minimizing any potential pain or distress.

Forty (40) male Wistar albino rats, with an average body weight of 180 ± 10 g, were obtained from the Animal House Colony of the National Research Centre in Cairo, Egypt. The animals were housed in the animal care facility at Faculty of Veterinary Medicine, Damanhour University, for two weeks under controlled environmental conditions, including a temperature of $21 \pm 2^{\circ}\text{C}$, a 12 h light/dark cycle, and humidity levels maintained at $60 \pm 10\%$. Throughout the acclimation period, the rats were provided with a standard rodent diet composed of 23% crude protein, 7% crude fiber, 3% crude fat, 8% acid-insoluble ash, 1–2.5% calcium, 0.5–1% phosphorus, and 9% sodium, along with free access to clean drinking water.

4.3. Experimental Design

After a two-week acclimatization period, forty (40) male albino Wistar rats were randomly assigned to four experimental groups ($n = 10$ per group) using online random

number generators (e.g., <https://www.graphpad.com/quickcalcs/randomize1/> (accessed on 1 May 2025)) according to ARRIVE guidelines. All rats, except those of the control group (negative CRT) ($n = 10$), were administered single intraperitoneal injections of 35 mg/kg STZ solution [39,98]. Following the injections, STZ-injected rats were kept on a high-fat diet (15% fats, 10% sucrose, 3% cholesterol, 3% sodium chloride, and 69% standard rat feed) for eight weeks [39] to induce type 2 diabetic testicular damage (T2DT) [98]. The control group received single IP injection of citrate buffer injections and were kept on a standard rodent diet. The rats were assigned as T2DT-positive if they exhibited FBG levels of at least 13.9 mmol/L (250 mg/dL), significantly increased 24 h proteinuria, and urine volumes 50% higher than those of the control group [99].

These T2DT rat groups were then again randomly divided, as mentioned previously (using the online random number generators <https://www.graphpad.com/quickcalcs/randomize1/> (accessed on 1 May 2025)) according to ARRIVE guidelines) into three experimental groups ($n = 10$ per group): the STZ group (positive CRT), the SEM-treated group subcutaneous inj. [22] (STZ + SEM 0.3 mg/kg) [100] and the SEM + papaya-treated group (STZ + SEM 0.3 mg/kg + 10% papaya pulp juice) [101]. A schematic illustration of the experimental design is presented in Figure 6.

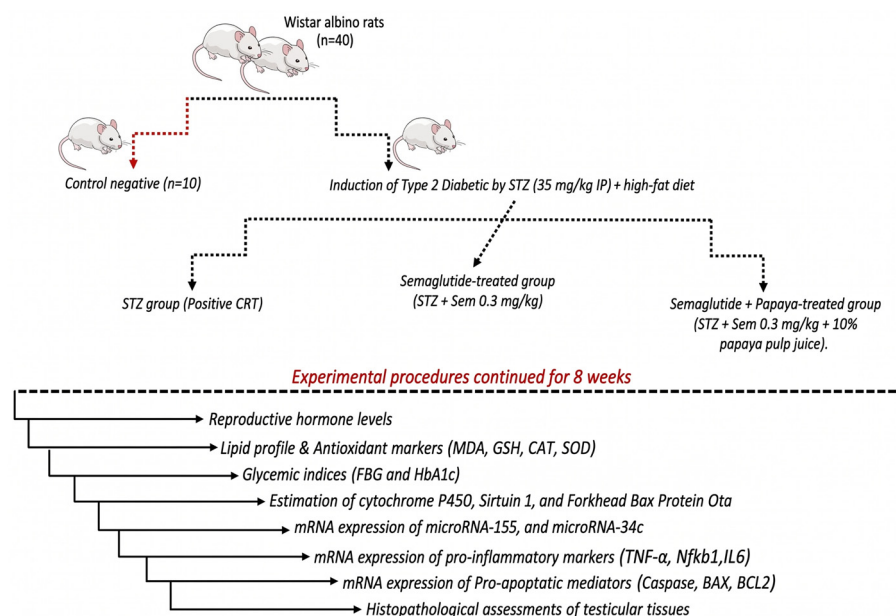


Figure 6. Schematic illustration of the experimental design (created using PowerPoint).

At the end of the experiment, the rats ($n = 10$ per group) were anesthetized with a combination of 87 mg/kg ketamine and 13 mg/kg xylazine. The anesthesia was initiated 10–15 min after the simultaneous injection and lasted for 15–30 min. The rats were then euthanized by cervical dislocation [102]. Blood samples were collected through cardiac puncture prior to performing the abdominal incision. Sera separation was achieved by centrifugation at 1008 g -force, and the samples were stored at -20°C for subsequent analysis of FBG, HbA1c, TAG, TC, HDL-C, LDL-C, VLDL, SHBG, testosterone, LH, and 17-beta estradiol. Both testes were immediately excised upon euthanasia, weighed, and divided into three sections. The first section was preserved at -80°C for gene expression analysis. The second section was homogenized and stored at -20°C for further ELISA assessments of SIRT1, FOXO1, and OS parameters. The third section was preserved in 10% buffered formalin for histological examination.

4.4. Evaluation of Sperm Parameters (Sperm Motility, Sperm Count and Sperm Morphology)

Following surgical excision, the left epididymis was immediately immersed in 100 μ L of saline pre-warmed to 37 °C. To obtain fresh semen, the epididymis was gently manipulated, and the sample was incubated for 30 min at 37 °C to allow sperm liquefaction. After incubation, a 20 μ L aliquot was carefully aspirated and examined under $\times 40$ magnification to assess both progressive and non-progressive motility (PR + NP) across ten microscopic fields, as previously described [103].

Sperm concentration in the fresh semen samples (run in duplicates) was determined using a Neubauer hemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany), and immotile sperm were defined as those showing no head or tail movement under microscopic examination. The number of spermatozoa in five counting squares was recorded and multiplied by 10,000 to calculate the total concentration per milliliter. To evaluate morphological abnormalities, smears were examined under a Leica DM 3000 light microscope (by Leica Microsystems, Wetzlar, Germany) set to $\times 400$ magnification, utilizing a $\times 40$ objective lens for precise observation [104].

4.5. Assessment of Diabetic, Lipid, and Fertility Profiles in Serum

The diabetic profile markers, including FBG and HbA1c, were assessed spectrophotometrically using commercial diagnostic kits from Human, Wiesbaden (Hessen), Germany. The lipids profile, including TAG, TC, HDL-C, LDL-C, and VLDL, was also evaluated using the same method. SHBG, testosterone (total/free/bioavailable), LH, and serum 17-beta estradiol levels were measured using ELISA kits sourced from My BioSource, San Diego, CA, USA.

4.6. Evaluation of Oxidative/Antioxidative Markers and Cytokines in Homogenized Testicular Tissues

After euthanasia, testicular tissues were immediately collected, weighed, homogenized, and centrifuged at 4000 rpm for 15 min using a buffer solution consisting of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 , and 2 mM KH_2PO_4 , pH 7.4. The supernatants were then analyzed to assess oxidative and antioxidative markers, including GSH, CAT, SOD, and MDA, following the manufacturer's guidelines with commercial kits. Additionally, ELISA kits were used to determine the levels of CYP2E1, SIRT1, and FOXO1 in the testicular tissues. All assays were conducted according to the manufacturers' protocols, and results were normalized to protein content as determined by the Bradford assay [105].

4.7. Assessment of Testicular mRNA Expression of miR-155, miR-34c, TNF- α , NF- κ B1, IL-6, CASP3, Bax, and Bcl-2

mRNA was isolated, and subsequently, cDNA synthesis was performed using the RevertAid™ Synthesis Kit as outlined by [96,106]. For gene expression analysis, β -actin was used as the housekeeping gene for TNF- α , NF- κ B1, IL-6, CASP3, Bax, and Bcl-2 genes analyzed per the published references [22,96,107]. The reactions were carried out in a thermal cycler (MyCycler, Bio-Rad, Munich, Germany) after preparing the master mix [22]. A total reaction volume of 25 μ L was used for each gene of interest, with polymerase chain reaction [108] primers ranging from 16 to 24 base pairs in length, as detailed in Table 6.

Expression levels of miR-34c-5p and miR-155-5p were quantified using TaqMan MicroRNA Assays ID 000428 and 002571, respectively, with U6 snRNA (Assay ID 001973) as the endogenous control. All assays were from Thermo Fisher Scientific (Waltham, MA, USA) and were performed according to the manufacturer's protocol using the TaqMan MicroRNA Reverse Transcription Kit and TaqMan Universal PCR Master Mix. The relative quantification of the target genes was determined using the $\Delta\Delta\text{CT}$ method [96,109] with the

reference gene U6 snRNA for miR-34c-5p and rno-miR-155-5p. Reactions were performed in triplicate.

Table 6. List of the primer sequences employed.

Gene	Primer [5' to 3']	TM	Accession No.
CASP3	F: GAAATTCAAGGGACGGGTCA R: TTCTTTGCATGGAAAGTGGC	57.81 57.18	NM_012922.2
Bax	F: GGCGAATTGGCGATGAACTG R: ATGGTTCTGATCAGCTCGGG	60.25 59.53	NM_017059.2
Bcl2	F: GTATGATAACCGGGAGATCG R: AGCCAGGAGAAATCAAACAG	54.95 55.63	NM_016993.1
Tnf- α	F: CCACGTCGTAGCAAACCAC R: TGGGTGAGGAGCACGTAGT	61.35 60.22	NM_012675.3
NF- κ B1	F: ACCTGAGTCTTCTGGACCGC R: CCAGCCTTCTCCCAAGAGTC	59.11 58.41	NM_001276711.1
IL-6	F: TATGTTCTCAGGGAGATCTTGGA R: GTGCATCATCGCTGTTTCATACA	60.4 59.7	NM_012589
β -actin	F: TCTTCCAGCCTTCCTTCCTG R: CACACAGAGTACTTGCGCTC	59.01 58.94	NM_031144.3

Accession number according to Nucleotide Sequences; RefSeq transcripts from the RGD Rate Genome Database and Thermo Fisher Scientific. (TM: melting temperature.)

4.8. Histopathologic Examination

Following euthanasia, the testes from five randomly selected rats from each experimental group (n = 10/group) were collected for histopathological evaluation. Samples were preserved in 10% buffered formalin for 48 h, then cleared in xylene, gradually dehydrated in ascending concentrations of ethyl alcohol, and subsequently embedded in paraffin. Sections of 4 μ m thicknesses were cut from each group using a rotary microtome (Leica RM2235, Leica Biosystems, Nussloch, Germany), mounted on glass slides, and stained with hematoxylin and eosin (H&E) [110]. The stained sections were examined under a trinocular light microscope (Leica DM500), and photomicrographs were captured using a digital camera (Leica EC3; Leica, Wetzlar, Germany) to assess tissue morphology and the extent of testicular injury. Histopathological evaluation was performed by a pathologist blinded to the treatment groups to minimize observer bias.

To calculate the Johnsen spermatogenesis scoring system to further strengthen the quantitative assessment of testicular recovery, each seminiferous tubule is graded from 10 (full spermatogenesis) to 1 (no germ cells) based on the most advanced germ cell present. From visual inspection of panels, approximate semi-quantitative scoring was performed.

4.9. Cheminformatics Studies of the Repurposed Semaglutide

4.9.1. In Silico Identification of Physicochemical Properties and Tox21

The SEM molecular formula is C187H291N45O59 per SMILES (<https://pubchem.ncbi.nlm.nih.gov/compound/56843331> (accessed on 18 October 2025)). The physicochemical parameters of SEM, the logarithm of the n-octanol/water distribution coefficient at pH = 7.4, logD7.4, and the acid–base dissociation constant, pKa, were determined using the ADMETlab 3.0 (<https://admetlab3.scbdd.com/server/evaluation> (accessed on 18 October 2025) online platform.

4.9.2. Comparative Toxicogenomics Database (CTD)

<https://ctdbase.org/> (accessed on 18 October 2025) [111] was used to identify SEM-interacting genes and pathways.

4.10. Bioinformatics Databases Analysis

4.10.1. Immune Cell Infiltration Functional Enrichment Analysis

SIRT1 and FOXO1 gene expression profiles were explored using the integrated human single-cell transcriptomic database “Human Universal Single Cell Hub” (HUSCH) (<http://husch.comp-genomics.org/#/search> (accessed on 18 October 2025)) [112] and the “Human Protein Atlas” from HPA database version 24.1 (<https://www.proteinatlas.org/> (accessed on 18 October 2025)) [113].

4.10.2. Protein–Protein Interaction and Functional Network Analysis

Gene interaction and pathway analyses were performed using SignaLink 3.0 (v3.1) (<http://signalink.org/> (accessed on 18 October 2025)) to investigate signaling pathway cross-talks, transcription factors, miRNAs, and regulatory enzymes [114]. Protein–protein interaction (PPI) networks were constructed using GeneMANIA (v3.6.0) (<http://www.genemania.org/> (accessed on 18 October 2025)) [115], STRING version 12.0 (<https://string-db.org/> (accessed on 18 October 2025)) [116] and STITCH version 5.0 (<http://stitch.embl.de/> (accessed on 18 October 2025)) [117]. In addition, the University of California Santa Cruz (UCSC) Genome Browser Gene Interactions tool [118] (<https://genome-euro.ucsc.edu/index.html> (accessed on 18 October 2025)) was used to identify gene interactions and associated biological pathways based on curated databases and text-mining evidence.

4.11. Statistical Analysis

The statistical analyses were performed using SPSS software version 20 (SPSS Inc., Richmond, VA, USA). Data are presented as mean $\pm$ standard error of the mean (SE of the mean). Prior to inferential testing, the assumptions required for parametric analysis were evaluated. The normality of data distribution was assessed using the Shapiro–Wilk test, while homogeneity of variances among groups was examined using Levene’s test. Independence of observations was ensured through the experimental design. Comparisons among multiple experimental groups were conducted using one-way analysis of variance (ANOVA). When a statistically significant overall effect was detected, Duncan’s multiple range post hoc test was applied for pairwise comparisons between treatment groups to identify specific differences. A p -value < 0.05 was considered statistically significant for all analyses.

5. Summary and Conclusions

In conclusion, both SEM alone and SEM combined with *C. papaya* juice attenuated several metabolic, reproductive, oxidative stress, molecular, and histopathological abnormalities in STZ-induced diabetic rats. Although the combination produced additional improvements in selected outcomes, it was not consistently superior to SEM alone. Moreover, the proposed involvement of apoptosis-related genes, NF- κ B1, and the miR-155/miR-34c–SIRT1/FOXO1 network is based on expression-level associations rather than direct functional evidence. Future studies should include papaya monotherapy, factorial dose-ranging designs, and functional assessment of apoptosis, NF- κ B activation, and FOXO1 activity before definitive mechanistic or translational conclusions can be drawn.

Figure 7 provides a schematic overview integrating bioinformatics, chemoinformatics, and experimental findings into a proposed molecular mechanism underlying the therapeutic effects of semaglutide and papaya.

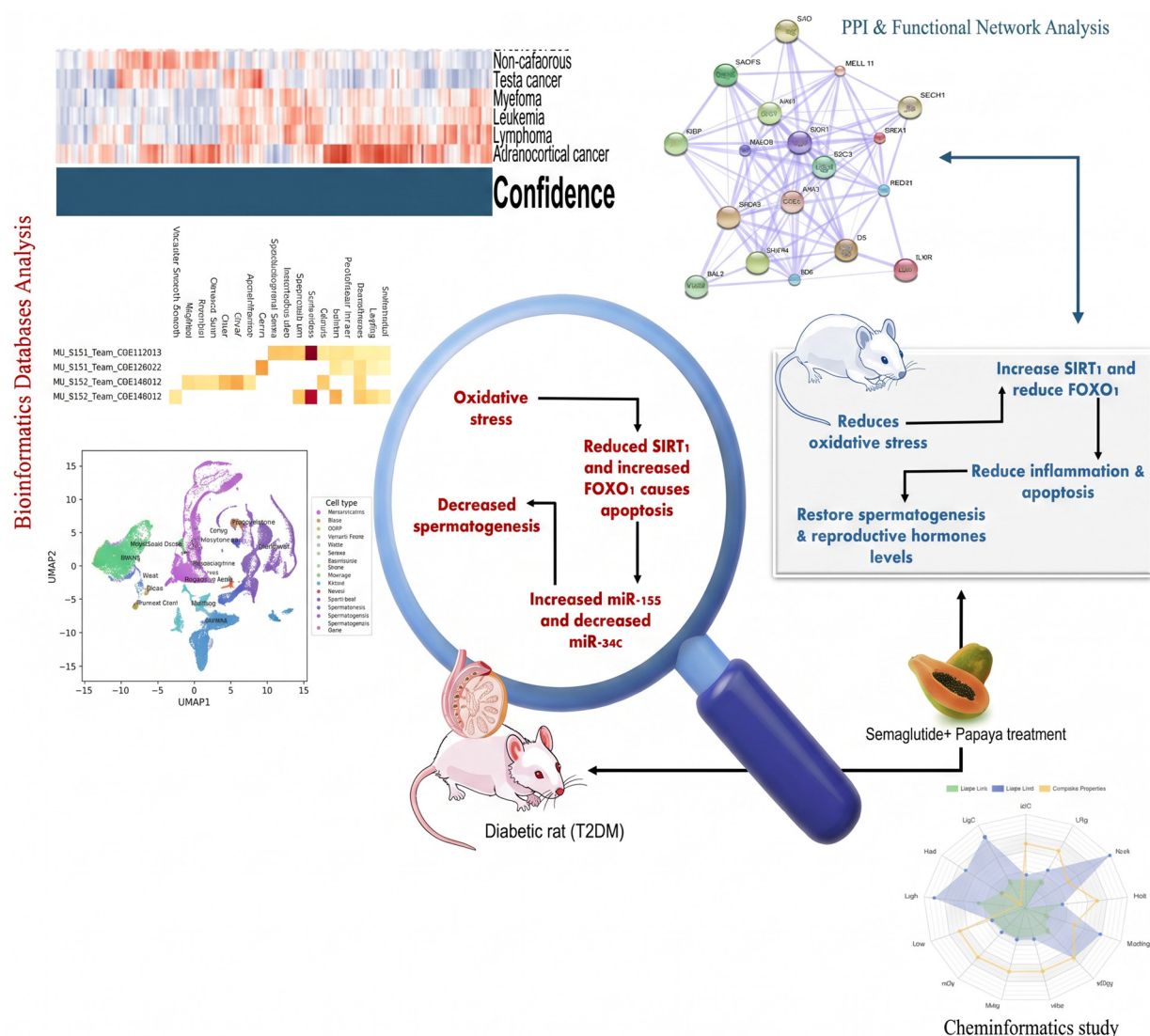


Figure 7. Schematic overview of the study integrating in silico bioinformatics and chemoinformatics analyses with experimental validation to investigate the protective effects of semaglutide (SEM) plus papaya against T2DM-induced male reproductive dysfunction. The workflow progresses from left (in silico analyses) to center (proposed disease mechanism) and right (therapeutic intervention and outcomes). Combined SEM and papaya treatment alleviates oxidative stress, restores the SIRT1/FOXO1 axis, reduces inflammation and apoptosis, and improves spermatogenesis and reproductive function in diabetic rats.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27156956/s1>.

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alization, supervision, writing—review and editing, and data curation; S.-M.H.: writing—review and editing, visualization, resources, and funding acquisition. N.M.H.: software, in silico analysis, validation, data curation, visualization, supervision, and writing, review and editing, submission till acceptance. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All procedures were conducted in accordance with institutional and national ethical standards for animal care and use. Ethical approval was obtained from the Hygiene and Preventive Medicine Research Committee at the Faculty of Veterinary Medicine, Damanhour University, Egypt (Approval No. DMU/VetMed-2025/016 on 4 May 2025). The study was conducted in alignment with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

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List of Abbreviations

ARRIVE: Animal Research: Reporting of In Vivo Experiments; ARs: androgen receptors; Bax: BCL2-Associated X Apoptosis Regulator; Bcl-2: B-cell lymphoma-2; CASP3: Caspase-3; CAT: catalase; Chol: total cholesterol; CYP2E1: cytochrome P450 2E1; DM: diabetes mellitus; ELISA: enzyme-linked immunosorbent assay; FBG: fasting blood glucose; FOXO1: forkhead box protein O1; GLP-1: glucagon-like peptide-1; GSH: reduced glutathione; H&E: hematoxylin and eosin; HbA1c: glycated hemoglobin; HDL-c: high-density lipoprotein cholesterol; IL-6: interleukin-6; LDL-c: low-density lipoprotein cholesterol; LH: luteinizing hormone; MDA: malondialdehyde; miRNAs, miR: microRNAs; NF- κ B1: nuclear factor kappa B subunit 1; OS: oxidative stress; PCR: polymerase chain reaction; ROS: reactive oxygen species; SEM: Semaglutide; SHBG: sex-hormone-binding globulin; SIRT1: sirtuin-1; SOD: superoxide dismutase; STZ: Streptozotocin; T2DM: type 2 diabetes mellitus; TAG: triacylglycerol; TNF- α : tumor necrosis factor-alpha; VLDL: very-low-density lipoprotein.

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