Supplementary information

Dietary phytoestrogen, diosgenin interrupts metabolism, physiology and reproduction of the Swiss albino mice: possible mode of action as an emerging environmental contaminant, endocrine disruptor and reproductive toxicant

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Table S1

Acute toxicity study on diosgenin in relation to body and organ weights, hepatic and renal function parameters, lipid profiles and lethal dose in male Swiss albino mice.

Body and organ weight and index	Experimental group						
	Control	Diosgenin 100 (mg/kg)	Diosgenin 200 (mg/kg)	Diosgenin 400 (mg/kg)	Diosgenin 600 (mg/kg)	Diosgenin 800 (mg/kg)	$F_{5,29}$ value
Body and organ weights							
Body weight (g)	30.26 ± 1.12a	29.86 ± 1.50a	29.66 ± 1.28a	21.36 ± 1.85b	19.50 ± 1.25b	17.93 ± 1.52b	16.029
Testis (mg)	108.12 ± 1.38a	107.45 ± 1.26a	106.24 ± 1.10a	99.12 ± 1.90b	97.46 ± 1.75b	96.30 ± 1.84b	11.815
Epididymis (mg)	58.94 ± 1.05a	57.62 ± 1.18a	58.05 ± 1.44a	49.25 ± 1.33b	48.92 ± 1.02b	47.82 ± 1.60b	16.729
Seminal vesicle (mg)	210.42 ± 1.27a	209.75 ± 1.35a	207.61 ± 1.18a	200.38 ± 1.91b	197.73 ± 1.26b	195.11 ± 1.18b	22.758
Vas deferens (mg)	30.10 ± 1.53a	30.02 ± 1.28a	29.85 ± 1.51a	24.01 ± 1.08b	21.47 ± 1.48b	20.65 ± 1.08b	11.229
Prostate (mg)	63.12 ± 1.90a	62.68 ± 1.32a	63.15 ± 1.48a	58.06 ± 1.55a	55.15 ± 1.40b	52.37 ± 1.45b	9.197
Liver (mg)	1400.16 ± 3.20a	1391.06 ± 4.20a	1387.16 ± 3.01a	1360.67 ± 5.16b	1332.90 ± 4.55c	1316.41 ± 4.75c	65.331
Kidney (mg)	200.75 ± 3.10a	202.50 ± 2.69a	197.30 ± 2.09a	190.71 ± 3.38ab	188.20 ± 2.50b	180.26 ± 2.82b	9.195
Adrenal (mg)	13.00 ± 0.52a	12.89 ± 0.47a	12.28 ± 0.35a	11.89 ± 0.45ab	10.05 ± 0.28b	9.89 ± 0.46b	10.273
GSI (%)	0.3573 ± 0.02a	0.3598 ± 0.02a	0.3582 ± 0.01a	0.4640 ± 0.01b	0.4998 ± 0.02bc	0.5557 ± 0.02c	24.774
Liver and kidney function profiles							
ALP (IU/L)	138.98 ± 2.16a	139.41 ± 2.06a	140.50 ± 1.85a	145.75 ± 1.30b	151.62 ± 1.20bc	155.82 ± 1.85bc	15.790
ALT (IU/L)	150.71 ± 1.72a	150.10 ± 1.08a	152.60 ± 1.15a	158.90 ± 2.61ab	165.30 ± 2.84bc	171.70 ± 2.49c	17.473
AST (IU/L)	173.68 ± 2.70a	176.15 ± 2.95a	177.75 ± 2.25a	185.90 ± 2.41b	190.60 ± 2.15b	194.68 ± 2.35b	11.798
AST/ALT ratio	0.8677 ± 0.01a	0.8521 ± 0.02a	0.8585 ± 0.03a	0.8548 ± 0.02a	0.8673 ± 0.03a	0.8820 ± 0.04a	0.1687
SC (µmol/L)	0.40 ± 0.16a	0.39 ± 0.05a	0.41 ± 0.03a	0.52 ± 0.05a	0.59 ± 0.09d	0.63 ± 0.05a	1.568
SU (µmol/L)	20.25 ± 0.68a	22.70 ± 0.95ab	24.50 ± 0.72b	32.47 ± 0.25c	38.41 ± 0.95d	41.45 ± 0.56d	146.69
BUN (mg/dL)	9.46 ± 0.56a	10.60 ± 0.81a	11.44 ± 0.75a	15.16 ± 0.95b	17.94 ± 0.82bc	19.36 ± 0.48c	30.319
B:C ratio	23.65 ± 1.82a	27.18 ± 1.47a	27.90 ± 1.62a	29.15 ± 1.55a	30.41 ± 1.95a	30.73 ± 1.38a	2.519
Lipid profiles							
TC (mg/dL)	30.65 ± 1.26a	34.15 ± 1.70a	32.84 ± 1.45a	40.82 ± 1.40b	44.20 ± 1.35bc	47.75 ± 1.29c	23.543
TG (mg/dL)	47.94 ± 1.82a	48.81 ± 1.72a	50.28 ± 1.75ab	55.62 ± 1.02bc	57.38 ± 1.25c	61.75 ± 1.53c	12.674
HDL (mg/dL)	82.36 ± 1.15a	80.60 ± 1.05a	78.55 ± 1.65a	72.46 ± 1.15b	67.82 ± 1.45bc	64.91 ± 1.28c	30.101
LDL (mg/dL)	30.20 ± 1.65a	32.72 ± 1.88a	31.70 ± 1.95a	43.60 ± 1.82b	51.36 ± 1.48c	58.46 ± 1.18c	48.832
Lethal dose 50 (LD_{50})							
LD_{50} (mg/kg)	95% Fiducial limit – Confidence interval				Slope	Intercept	R^2
546.265	Lower limit: 412.961		Upper limit: 722.598		3.540	-4.689	0.989

Values are expressed as mean ± standard error mean (n = 5/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $P < 0.0001$ and with similar letters indicate that treatment groups are not statistically significant at $P > 0.05$.

AST:ALT ratio = AST/ALT; Blood urea nitrogen = Serum urea × 0.467; B:C ratio: BUN/Creatinine level.

GSI: Gonado-somatic index; **ALP**: Serum alkaline phosphatase; **ALT**: Serum alanine transaminase; **AST**: Serum aspartate aminotransferase; **SC**: Serum creatinine; **SU**: Serum urea; **BUN**: Blood urea nitrogen; **B:C ratio**: BUN:Creatinine ratio; **TC**: Serum total cholesterol; **TG**: Serum triglycerides; **HDL**: Serum high density lipoprotein; **LDL**: Serum low density lipoprotein.

Table S2

Acute toxicity study on diosgenin in relation to body and organ weights, hepatic and renal function parameters, lipid profiles and lethal dose in female Swiss albino mice.

Body and organ weight and index	Experimental group						
	Control	Diosgenin 100 (mg/kg)	Diosgenin 200 (mg/kg)	Diosgenin 400 (mg/kg)	Diosgenin 600 (mg/kg)	Diosgenin 800 (mg/kg)	$F_{5,29}$ value
Body and organ weights							
Body weight (g)	31.08 ± 0.92a	32.18 ± 0.49a	30.50 ± 0.56a	25.66 ± 0.72b	23.38 ± 0.26bc	22.70 ± 0.18c	51.767
Ovary (mg)	58.62 ± 0.90a	57.35 ± 0.74a	56.28 ± 0.52a	50.35 ± 0.41b	47.46 ± 0.45c	45.25 ± 0.40c	87.743
Uterus (mg)	902.16 ± 1.28a	904.75 ± 1.35a	900.77 ± 1.85a	890.62 ± 1.06b	87.45 ± 1.02c	880.28 ± 1.26d	61475
Liver (mg)	1258.95 ± 3.88a	1262.62 ± 4.05a	1260.26 ± 3.45a	1191.38 ± 3.85b	1102.61 ± 4.32c	1008.50 ± 2.16d	810.14
Kidney (mg)	275.25 ± 1.38a	272.05 ± 1.80a	270.28 ± 1.71a	262.25 ± 1.95b	257.05 ± 1.82bc	250.58 ± 1.46c	31.899
Adrenal (mg)	12.01 ± 0.85a	12.32 ± 0.76a	12.90 ± 0.65a	10.12 ± 0.38b	9.31 ± 0.05b	8.85 ± 0.16b	9.236
GSI (%)	0.1886 ± 0.03a	0.1782 ± 0.04a	0.1845 ± 0.03a	0.1962 ± 0.04a	0.2030 ± 0.04a	0.1993 ± 0.04a	0.06576
Hepato-renal function parameters							
ALP (IU/L)	78.25 ± 1.02a	80.48 ± 1.75ab	79.69 ± 1.15a	86.36 ± 1.18bc	92.46 ± 1.90c	99.84 ± 1.63d	33.342
ALT (IU/L)	41.94 ± 1.73a	40.35 ± 1.86a	42.60 ± 1.31a	50.64 ± 1.75b	54.60 ± 1.26bc	59.28 ± 1.57c	23.699
AST (IU/L)	23.45 ± 1.79a	22.92 ± 1.62a	24.25 ± 1.39a	30.18 ± 1.55ab	34.26 ± 1.20bc	39.40 ± 1.73c	18.705
AST/ALT ratio	0.5591 ± 0.18a	0.5680 ± 0.11a	0.5692 ± 0.09a	0.5960 ± 0.20a	0.6275 ± 0.18a	0.6646 ± 0.16a	0.06813
SU (µmol/L)	25.14 ± 1.34a	27.66 ± 1.23a	28.58 ± 1.05a	36.85 ± 1.49bc	39.72 ± 1.80c	41.59 ± 1.18c	25.911
SC (µmol/L)	0.12 ± 0.02a	0.15 ± 0.02a	0.18 ± 0.03a	0.39 ± 0.01b	0.48 ± 0.03bc	0.57 ± 0.04c	50.609
BUN (mg/dL)	11.74 ± 1.86a	12.92 ± 0.79a	13.35 ± 0.56a	17.21 ± 0.84b	18.55 ± 0.95b	19.42 ± 0.79b	9.594
B:C ratio	21.00 ± 0.96a	22.74 ± 0.82a	23.45 ± 0.21a	28.88 ± 0.66b	29.56 ± 0.73b	29.22 ± 0.36b	32.123
Lipid profiles							
TC (mg/dL)	49.74 ± 0.71a	50.28 ± 0.38a	52.55 ± 0.50a	60.35 ± 0.95b	66.31 ± 0.59c	69.38 ± 0.84d	151.98
TG (mg/dL)	56.77 ± 0.06a	58.51 ± 0.72a	62.05 ± 0.62b	70.25 ± 0.50c	76.20 ± 0.22d	81.40 ± 0.17e	488.40
HDL (mg/dL)	85.88 ± 1.02a	81.05 ± 1.60ab	79.02 ± 1.82b	60.76 ± 1.21c	55.07 ± 1.36cd	49.28 ± 1.61d	110.46
LDL (mg/dL)	38.31 ± 0.40a	42.05 ± 0.89b	46.88 ± 0.16c	54.81 ± 0.95d	62.50 ± 0.72e	66.41 ± 0.84f	246.73
Lethal dose 50 (LD_{50})							
LD_{50} (mg/kg)	95% Fiducial limit – Confidence interval				Slope	Intercept	R^2
538.724	Lower limit: 443.493		Upper limit: 654.404		5.682	-10.520	0.993

Values are expressed as mean ± standard error mean (n = 5/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $P < 0.0001$ and with similar letters indicate that treatment groups are not statistically significant at $P > 0.05$.

AST:ALT ratio = AST/ALT; Blood urea nitrogen = Serum urea × 0.467; B:C ratio: BUN/Creatinine level.

GSI: Gonado-somatic index; **ALP**: Serum alkaline phosphatase; **ALT**: Serum alanine transaminase; **AST**: Serum aspartate aminotransferase; **SC**: Serum creatinine; **SU**: Serum urea; **BUN**: Blood urea nitrogen; **B:C ratio**: BUN:Creatinine ratio; **TC**: Serum total cholesterol; **TAC**: Serum triglycerides; **HDL**: Serum high density lipoprotein; **LDL**: Serum low density lipoprotein.

Table S3

Long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) on body and organ weights of male and female Swiss albino mice.

Body and organ weight and index	Experimental group					
	Control	Diosgenin 10 (mg/kg)	Diosgenin 50 (mg/kg)	Diosgenin 100 (mg/kg)	Diosgenin 200 (mg/kg)	$F_{4,24}$ value
Male Swiss albino mice						
Body weight (g)	34.38 ± 0.28a	29.62 ± 0.68b	26.22 ± 0.31b	22.12 ± 1.39b	21.26 ± 1.22b	25.53
Testis (mg)	111.94 ± 0.81a	109.52 ± 0.42a	95.42 ± 0.76b	90.25 ± 0.25b	86.75 ± 0.80c	15.08
Epididymis (mg)	59.12 ± 0.86a	48.21 ± 0.54b	47.80 ± 0.71b	40.96 ± 0.53c	36.10 ± 0.27c	15.97
Seminal vesicle (mg)	215.78 ± 1.70a	193.90 ± 1.24b	189.80 ± 0.88b	148.76 ± 1.60c	76.46 ± 1.89d	23.52
Vas deferens (mg)	29.51 ± 1.01a	20.28 ± 0.87b	20.30 ± 0.95b	17.94 ± 0.75b	15.68 ± 1.05b	21.24
Prostate (mg)	64.96 ± 0.66a	44.05 ± 0.71b	46.98 ± 2.47b	45.62 ± 2.39b	33.26 ± 0.87c	32.38
Liver (mg)	1403.36 ± 4.39a	1163.10 ± 3.08b	1154.74 ± 3.44b	1044.90 ± 4.10b	917.30 ± 4.71c	39.87
Kidney (mg)	207.38 ± 5.80a	204.40 ± 4.83a	182.40 ± 5.81b	173.03 ± 7.65b	152.62 ± 3.85c	15.77
Adrenal (mg)	13.78 ± 0.93a	8.17 ± 0.22b	8.86 ± 0.73b	7.34 ± 0.56b	6.58 ± 0.36b	21.35
Gonado-somatic index (GSI, %)	0.3256 ± 0.01a	0.3698 ± 0.02a	0.3639 ± 0.01a	0.4080 ± 0.01b	0.4015 ± 0.02b	4.971
Female Swiss albino mice						
Body weight (g)	33.53 ± 0.51a	30.21 ± 0.32b	27.36 ± 0.28c	25.35 ± 0.17d	24.05 ± 0.45d	108.67
Ovary (mg)	60.66 ± 0.34a	58.87 ± 0.34b	50.31 ± 0.66c	36.98 ± 0.12d	25.75 ± 0.27e	1474
Uterus (mg)	915.74 ± 0.59a	840.21 ± 0.19b	537.52 ± 0.15c	394.40 ± 1.63d	326.86 ± 1.54e	6396
Liver (mg)	1265.53 ± 3.87a	1256.21 ± 2.83a	1054.22 ± 3.98b	1098.45 ± 4.08b	890.61 ± 2.87c	49.923
Kidney (mg)	278.75 ± 4.65a	261.74 ± 4.83a	237.44 ± 5.11b	198.32 ± 5.44c	155.96 ± 4.99d	98.178
Adrenal (mg)	11.55 ± 0.56a	10.45 ± 0.36a	9.72 ± 1.45a	7.77 ± 0.26b	5.16 ± 0.82b	26.688
Gonado-somatic index (GSI, %)	0.1809 ± 0.02a	0.1949 ± 0.02a	0.1839 ± 0.01a	0.1459 ± 0.01a	0.1071 ± 0.01b	5.899

Values are expressed as mean ± standard error mean (n = 5/sex/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $P < 0.0001$ and with similar letters indicate that treatment groups are not statistically significant at $P > 0.05$. GSI = [(gonad weight/body weight) × 100].

Table S4

Long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) on hepatic and renal function, lipid profiles, testicular testosterone levels, testis damage score, adrenal gland volume and immunoreactivity ratio in the male Swiss albino mice.

Parameters	Treatment Groups (mg/kg)					
	Control	Diosgenin 10 mg/kg	Diosgenin 50 mg/kg	Diosgenin 100 mg/kg	Diosgenin 200 mg/kg	$F_{4,24}$ value
Liver function assay						
Serum total protein (g/L)	138.60 ± 2.95a	130.02 ± 1.84b	124.44 ± 1.08b	115.52 ± 1.66c	96.75 ± 1.38d	71.243
Serum alkaline phosphatase (ALP, IU/L)	140.62 ± 4.43a	151.30 ± 4.32ab	158.42 ± 6.24b	190.85 ± 3.90c	201.31 ± 5.87c	27.097
Serum alanine transaminase (ALT, IU/L)	153.80 ± 2.16a	158.26 ± 3.43a	166.37 ± 1.14a	195.61 ± 2.16b	228.33 ± 3.12b	38.592
Serum aspartate aminotransferase (AST, IU/L)	177.4 ± 5.87a	189.23 ± 6.55a	205.88 ± 8.77a	265.14 ± 5.26b	357.43 ± 11.9c	84.793
AST/ALT ratio	1.15 ± 0.03a	1.19 ± 0.04a	1.23 ± 0.02a	1.35 ± 0.03b	1.56 ± 0.02b	32.595
Kidney function assay						
Serum creatinine (SC, µmol/L)	0.38 ± 0.12a	0.41 ± 0.08a	0.54 ± 0.06b	0.67 ± 0.11c	0.83 ± 0.14d	3.122
Serum urea (SU, µmol/L)	21.71 ± 1.12a	33.21 ± 1.45b	39.46 ± 2.01bc	43.89 ± 1.95c	50.66 ± 2.51d	34.656
Blood urea nitrogen (BUN, mg/dL)	23.87 ± 1.12a	32.45 ± 3.65a	55.98 ± 2.76b	61.87 ± 4.77b	83.24 ± 5.87c	35.599
BUN/Creatinine ratio (B:C)	62.81 ± 2.56a	79.14 ± 4.13a	103.66 ± 1.87b	92.34 ± 4.71b	100.28 ± 5.56b	17.569
Serum lipid profiles						
Serum total cholesterol (TC, mg/dL)	35.75 ± 2.05a	39.20 ± 3.45ab	48.13 ± 1.54bc	53.62 ± 2.65cd	66.81 ± 3.14d	21.581
Serum triglycerides (TG, mg/dL)	46.88 ± 3.02a	49.36 ± 2.54a	53.20 ± 3.42a	69.53 ± 3.87b	88.07 ± 1.76c	33.093
Serum high density lipoprotein (HDL, mg/dL)	80.54 ± 1.21a	80.05 ± 1.11a	72.95 ± 2.25a	52.31 ± 1.69b	50.54 ± 2.01b	74.666
Serum low density lipoprotein (LDL, mg/dL)	31.56 ± 3.69a	39.78 ± 1.48a	50.68 ± 1.58b	66.98 ± 3.45c	69.28 ± 2.65c	36.642
Testicular testosterone level and testis damage score						
Testicular testosterone (pg/mg protein)	45.21 ± 1.26a	38.01 ± 2.04b	32.33 ± 2.14b	29.15 ± 2.26bc	24.86 ± 2.90c	23.579
Testis tissue damage score	0a	1.01 ± 0.06b	1.85 ± 0.08b	3.56 ± 0.45c	3.78 ± 0.41c	116.17
Immunoreactivity ratio – point count method - Testis						
PCNA immunoreactivity ratio	23.71 ± 0.88a	15.22 ± 0.65b	9.75 ± 0.65c	5.80 ± 0.76 d	3.46 ± 0.14d	149.01
BAX immunoreactivity ratio	5.76 ± 0.65 a	17.87 ± 1.36 b	28.9 ± 3.66c	55.77 ± 2.87d	75.09 ± 3.62e	108.42
BCL-2 immunoreactivity ratio	47.80 ± 2.56a	45.43 ± 1.46a	38.98 ± 3.87a	22.51 ± 2.84b	15.41 ± 1.03c	31.394
BAX/BCL-2 ratio	0.12 ± 0.02a	0.39 ± 0.05b	0.72 ± 0.04c	2.47 ± 0.02d	4.87 ± 0.06e	2325.5
Active Caspase-3 immunoreactivity ratio	12.35 ± 0.28a	18.09 ± 0.95b	25.40 ± 0.60c	33.50 ± 0.72d	43.75 ± 0.82e	305.45
StAR immunoreactivity ratio	84.62 ± 1.45a	77.08 ± 2.54a	61.92 ± 1.66b	50.23 ± 2.87c	27.81 ± 1.82d	111.15
3β-HSD immunoreactivity ratio	28.33 ± 1.65a	25.98 ± 1.09a	20.44 ± 1.87bc	15.76 ± 0.46c	9.97 ± 0.81d	33.817
LHR immunoreactivity ratio	47.83 ± 2.76a	41.98 ± 2.69ab	35.78 ± 2.31bc	29.05 ± 1.22c	12.92 ± 0.69d	40.951
Aromatase immunoreactivity ratio	18.45 ± 1.36a	25.80 ± 1.95b	36.73 ± 1.48c	48.28 ± 1.60d	65.50 ± 1.18e	147.31

Adrenal gland histology - volume estimation[$]						
Adrenal cortex volume (AC, mm^3)	19.86 ± 0.05a	18.95 ± 0.26a	16.92 ± 0.44b	14.82 ± 0.66c	13.20 ± 0.18d	52.734
Adrenal medulla volume (AM, mm^3)	5.05 ± 0.09a	7.16 ± 0.90ab	8.52 ± 0.73bc	9.10 ± 0.41bc	10.52 ± 0.62c	11.304
Zona glomerulosa volume (ZG, mm^3)	3.55 ± 0.41a	2.59 ± 0.85a	2.38 ± 0.28a	2.05 ± 0.02a	2.01 ± 0.05a	3.234
Zona fasciculata volume (ZF, mm^3)	10.17 ± 0.38a	9.01 ± 0.05a	8.75 ± 0.15ab	7.82 ± 0.31bc	6.66 ± 0.90c	9.664
Zona reticularis volume (ZR, mm^3)	5.45 ± 0.62a	4.35 ± 0.20ab	3.51 ± 0.06bc	2.44 ± 0.20cd	2.10 ± 0.25d	17.870

Values are expressed as mean ± standard error mean (n = 5/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $P < 0.0001$ and with similar letters indicate that treatment groups are not statistically significant at $P > 0.05$.

[$] n = 10 fields/treatment group and $F_{4,49}$.

Testis tissue damage score: Normal: score - 0; < 25% damage: score 1; 25 - 50% damage: score 2; 50 - 75% damage: score 3; 75 - 100% damage: score 4.

PCNA/BCL-2/StAR/LHR/3β-HSD immunoreactivity ratio = {[Positively reacted cells]/[total number of cells]×100}.

PCNA: Proliferating cell nuclear antigen; BCL-2: B-cell lymphoma 2; BAX: Bcl-2-associated X protein; StAR: Steroidogenic acute regulatory protein; 3β-HSD: 3β-hydroxysteroid dehydrogenase; LHR: Luteinizing hormone receptor.

Table S5

Long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) on hepatic and renal function, lipid profiles, reproductive hormone levels, oxidative stress markers and adrenal gland volume in the female Swiss albino mice.

Parameters	Experimental group					
	Control	Diosgenin 10 mg/kg	Diosgenin 50 mg/kg	Diosgenin 100 mg/kg	Diosgenin 200 mg/kg	$F_{4,24}$ value
Liver function assay						
Serum total protein (g/L)	127.35 ± 1.13a	122.65 ± 1.64ab	118.44 ± 1.08b	112.55 ± 1.64c	100.36 ± 1.05d	60.945
Serum alanine transaminase (ALT, IU/L)	40.16 ± 1.66a	44.52 ± 1.28a	56.58 ± 1.62b	58.15 ± 1.45b	67.22 ± 1.73c	49.302
Aspartate aminotransferase (AST, IU/L)	26.38 ± 1.35a	36.38 ± 1.15b	56.25 ± 1.04c	85.56 ± 1.22d	98.18 ± 1.18e	670.39
AST/ALT ratio	0.65 ± 0.12a	0.81 ± 0.03a	0.99 ± 0.05a	1.47 ± 0.03b	1.46 ± 0.02b	36.805
Serum alkaline phosphatase (ALP, IU/L)	80.15 ± 1.12a	86.28 ± 1.01ab	84.12 ± 1.45ab	91.45 ± 1.22b	122.68 ± 1.45c	30.617
Kidney function assay						
Serum urea (mg/dL)	27.45 ± 0.18a	35.85 ± 1.42b	47.62 ± 1.54c	58.85 ± 1.34d	62.28 ± 1.15d	145.76
Serum creatinine (mg/dL)	0.14 ± 0.04a	0.21 ± 0.08a	0.33 ± 0.04ac	0.52 ± 0.02a	0.75 ± 0.06b	22.518
Blood urea nitrogen (BUN, mg/dL)	33.91 ± 1.22a	37.05 ± 0.93a	45.87 ± 2.73a	62.41 ± 1.87b	69.60 ± 3.85b	43.063
BUN/Creatinine ratio	242.21 ± 5.87a	176.42 ± 3.91b	139.00 ± 5.35c	120.01 ± 2.57c	92.8 ± 3.99d	166.13
Serum lipid profiles						
Serum total cholesterol (mg/dL)	48.04 ± 3.15a	56.66 ± 2.28a	71.72 ± 2.83b	76.10 ± 1.39b	82.26 ± 2.08b	34.212
Serum triglycerides (mg/dL)	55.20 ± 2.18a	63.59 ± 1.85a	71.46 ± 2.75a	78.91 ± 3.22b	90.25 ± 2.48b	28.444
Serum high density lipoprotein (HDL, mg/dL)	88.37 ± 1.70a	75.28 ± 1.20b	69.35 ± 2.05b	53.10 ± 1.66c	48.19 ± 2.18c	83.981
Serum low density lipoprotein (LDL, mg/dL)	35.21 ± 1.90a	48.26 ± 1.84b	57.27 ± 1.40c	67.26 ± 2.16d	76.72 ± 2.30e	69.099
Reproductive hormone levels						
Serum estradiol (pg/mL)	19.46 ± 0.29a	17.99 ± 0.56ab	16.22 ± 0.54bc	15.15 ± 0.45c	14.76 ± 0.33c	19.582
Serum luteinizing hormone (LH, pg/mL)	2136.35 ± 10.76a	2056.82 ± 12.91b	1878.36 ± 15.87c	1529.48 ± 10.44d	1266.90 ± 15.5e	762.69
Serum follicle stimulating hormone (FSH, IU/L)	17.36 ± 1.43a	15.77 ± 1.08a	12.87 ± 1.92b	9.56 ± 0.45b	7.44 ± 0.22b	12.004
Serum progesterone (ng/mL)	225.58 ± 7.87a	219.6 ± 5.76a	178.55 ± 3.09b	153.4 ± 5.98b	112.5 ± 4.17c	70.417

Oxidative stress markers						
Malondialdehyde (nmol/mg)	5.56 ± 0.44a	10.77 ± 1.33a	17.62 ± 2.09b	34.87 ± 3.21b	47.24 ± 2.77c	62.271
Catalase (µmol of H_2O_2 consumed/ min/ mg of protein)	10.55 ± 0.44a	9.56 ± 0.40a	7.59 ± 0.34b	5.93 ± 0.24b	3.87 ± 0.55c	44.007
Superoxide dismutase (U/ mg of protein)	39.94 ± 1.12a	27.76 ± 0.49b	23.02 ± 1.33b	15.07 ± 0.99c	10.84 ± 0.78c	133.93
Glutathione S transferase (µmol / mg of protein)	5.49 ± 0.33a	4.98 ± 0.23a	3.76 ± 0.21b	3.33 ± 0.06b	2.05 ± 0.45c	22.631
Reduced glutathione(µmol / mg of protein)	12.77 ± 0.65a	10.11 ± 0.44b	7.88 ± 0.54c	8.19 ± 0.66c	5.05 ± 0.23d	29.328
Adrenal gland histology - volume estimation[$]						
Adrenal cortex volume (AC, mm^3)	20.05 ± 0.72a	18.65 ± 0.81ab	16.28 ± 0.53bc	14.46 ± 0.29cd	13.72 ± 0.47d	20.464
Adrenal medulla volume (AM, mm^3)	5.38 ± 0.18a	7.56 ± 0.36ab	8.79 ± 0.42bc	9.54 ± 0.81bc	10.65 ± 0.72c	13.508
Zona glomerulosa volume (ZG, mm^3)	3.61 ± 0.85a	3.01 ± 0.62a	2.73 ± 0.39a	2.53 ± 0.05a	2.18 ± 0.28a	1.084
Zona fasciculata volume (ZF, mm^3)	10.85 ± 0.13a	9.55 ± 0.60ab	8.62 ± 0.42abc	7.34 ± 0.85bc	6.95 ± 0.69c	7.318
Zona reticularis volume (ZR, mm^3)	5.28 ± 0.75a	4.19 ± 0.86ab	3.51 ± 0.38ab	2.94 ± 0.72ab	2.65 ± 0.05b	2.828
Immunoreactivity ratio – point count method - Ovary						
PCNA immunoreactivity ratio	50.48 ± 0.90a	36.45 ± 0.12b	27.20 ± 0.31c	20.25 ± 0.58 d	18.75 ± 0.30d	639.05
Visfatin immunoreactivity ratio	30.18 ± 0.86a	20.49 ± 0.15b	11.60 ± 0.39c	9.05 ± 0.05d	4.35 ± 0.07e	570.84

Values are expressed as mean ± standard error mean (n = 5/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $p < 0.05$ and with similar letters indicate that treatment groups are not statistically significant at $p > 0.05$.

[$] n = 10 fields/treatment group and $F_{4,49}$; AST:ALT ratio = AST/ALT; Blood urea nitrogen = Serum urea × 0.467.

PCNA/Visfatin immunoreactivity ratio = {[Positively reacted cells]/[total number of cells]×100}.

Table S6

Long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) on the estrous cycle of female mice.

Parameter	Experimental group						
	Control	Diosgenin 10 mg/kg	Diosgenin 50 mg/kg	Diosgenin 100 mg/kg	Diosgenin 200 mg/kg	$F_{4,24}$ value	*p* value
Estrous cycle							
Cycle length (days)	4.86 ± 0.12a	4.92 ± 0.14a	5.31 ± 0.15a	5.46 ± 0.20a	6.77 ± 0.15b	24.257	0.0001
No. of regular cycle	6.00 ± 0.10a	5.00 ± 0.21b	3.00 ± 0.15c	2.00 ± 0.10d	1.00 ± 0.20e	169.83	0.0001
No. of Irregular cycles	2.00 ± 0.12a	3.00 ± 0.01b	5.00 ± 0.06c	6.00 ± 0.11d	7.00 ± 0.19e	324.28	0.0001
Duration of the estrous phase (days)							
Proestrus	4.83 ± 0.16a	4.09 ± 0.23a	3.55 ± 0.13b	3.33 ± 0.21b	2.16 ± 0.16c	22.524	0.0001
Estrus	5.34 ± 0.44a	4.66 ± 0.32a	4.23 ± 0.12a	3.88 ± 0.38b	2.36 ± 0.41b	10.552	0.0001
Metestrus	2.00 ± 0.12a	2.90 ± 0.05b	3.67 ± 0.12c	3.83 ± 0.33d	4.16 ± 0.47d	101.30	0.0001
Diestrus	8.66 ± 0.21a	8.97 ± 0.32a	9.87 ± 0.21b	10.33 ± 0.25b	11.00 ± 0.21c	15.598	0.0001
Diestrus index	9.66 ± 0.12a	9.96 ± 0.11a	10.96 ± 0.11b	11.47 ± 0.05c	12.22 ± 0.03d	186.22	0.0001

Values are expressed in mean ± standard error mean (n = 5/treatment group). D = diosgenin. One-way ANOVA with Tukey post hoc multiple comparison tests. Alphabet between columns signify that mean ± standard error mean with different letters differ significantly at $P < 0.0001$ while mean ± standard error mean with the same letter do not differ significantly at $P < 0.0001$.

Diestrus index = Number of days with clear diestrus smear /Total duration of treatment (Days) × 100.

Table S7

One way ANOVA analysis showing the interaction among liver and kidney function parameters and alterations in the estrous cyclicity upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	STP	**ALT**	**AST**	**AAR**	**Urea**	**Creat**	**BUN**	**BCR**	**Eslen**	**RC**	**IRRC**	**PEst**	**Est**	**Mest**	**Dest**	**DEI**
STP	0	*0.0001*	*0.0006*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0820*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*
ALT	7.951	0	*1*	*0.0016*	*1*	*0.0013*	*1*	*0.0001*	*0.0060*	*0.0033*	*0.0046*	*0.0035*	*0.0040*	*0.0032*	*0.0197*	*0.0263*
AST	7.039	0.9126	0	*0.0002*	*0.996*	*0.0002*	*0.9998*	*0.0001*	*0.0007*	*0.0004*	*0.0006*	*0.0004*	*0.0005*	*0.0004*	*0.0025*	*0.0035*
AAR	14.55	6.601	7.513	0	*0.0122*	*1*	*0.0047*	*0.0001*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*0.9999*
Urea	8.825	0.8737	1.786	5.727	0	*0.0101*	*1*	*0.0001*	*0.0388*	*0.0228*	*0.0312*	*0.0240*	*0.0274*	*0.0223*	*0.1065*	*0.1342*
Creat	14.64	6.687	7.6	0.0866	5.814	0	*0.0038*	*0.0001*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*0.9999*
BUN	8.401	0.4495	1.362	6.151	0.4242	6.238	0	*0.0001*	*0.0162*	*0.0091*	*0.0128*	*0.0097*	*0.0111*	*0.0089*	*0.0490*	*0.0636*
BCR	4.777	12.73	11.82	19.33	13.6	19.42	13.18	0	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Eslen	14	6.046	6.959	0.5543	5.173	0.641	5.597	18.78	0	*1*	*1*	*1*	*1*	*1*	*1*	*1*
RC	14.26	6.307	7.22	0.2936	5.433	0.3802	5.857	19.04	0.2607	0	*1*	*1*	*1*	*1*	*1*	*1*
IRRC	14.11	6.155	7.068	0.4452	5.282	0.5318	5.706	18.88	0.1091	0.1516	0	*1*	*1*	*1*	*1*	*1*
PEst	14.23	6.283	7.195	0.3178	5.409	0.4045	5.833	19.01	0.2365	0.0242	0.1273	0	*1*	*1*	*1*	*1*
Est	14.17	6.219	7.132	0.3813	5.346	0.4679	5.77	18.95	0.1731	0.0876	0.0639	0.0634	0	*1*	*1*	*1*
Mest	14.27	6.318	7.231	0.2825	5.444	0.3691	5.869	19.05	0.2719	0.0111	0.1627	0.0353	0.0987	0	*1*	*1*
Dest	13.45	5.503	6.415	1.098	4.629	1.184	5.053	18.23	0.5435	0.8042	0.6526	0.7799	0.7165	0.8153	0	*1*
DEI	13.32	5.365	6.278	1.235	4.492	1.322	4.916	18.09	0.6809	0.9416	0.79	0.9174	0.854	0.9527	0.1374	0

Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons.

F value is shown in regular font while *P*-value is shown in italics font. Statistically significant values between variables are shown as highlighted in italics ($P < 0.05$).

One way ANOVA: $F_{15,64} = 33.37$ and $P = 1.72E\text{-}24$.

Levene's test for homogeneity of variance: $P = 3.995E\text{-}11$.

Welch F test: $F = 91.97$ and $P = 2.596E\text{-}17$.

Abbreviations: **STP:** Serum total protein; **ALT:** Serum alanine transaminase; **AST:** Aspartate aminotransferase; **AAR:** ALT/AST ratio; **Creat:** Creatinine; **BUN:** Blood urea nitrogen; **BCR:** BUN/Creat ratio; **Eslen:** Estrous cycle length; **RC:** Regular cycle; **IRRC:** Irregular cycle; **PEst:** Proestrous; **Est:** Estrous; **Mest:** Metestrous; **Dest:** Diestrous; **DEI:** Diestrous index.

Table S8

Linear correlation analysis showing the interaction among liver and kidney function parameters and alterations in the estrous cyclicity upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	STP	**ALT**	**AST**	**AAR**	**Urea**	**Creat**	**BUN**	**BCR**	**Eslen**	**RC**	**IRRC**	**PEst**	**Est**	**Mest**	**Dest**	**DEI**
STP	0	*0.0126*	*0.0102*	*0.0339*	*0.0232*	*0.0006*	*0.0093*	*0.0342*	*0.0064*	*0.0164*	*0.0164*	*0.0023*	*0.0006*	*0.0494*	*0.0072*	*0.0070*
ALT	-0.9518	0	*0.0104*	*0.0323*	*0.0070*	*0.0122*	*0.0169*	*0.0107*	*0.0381*	*0.0012*	*0.0012*	*0.0055*	*0.0142*	*0.0099*	*0.0008*	*0.0009*
AST	-0.9581	0.9575	0	*0.0019*	*0.0018*	*0.0034*	*0.0002*	*0.0223*	*0.0522*	*0.0032*	*0.0032*	*0.0176*	*0.0228*	*0.0286*	*0.0022*	*0.0022*
AAR	-0.9064	0.9095	0.9862	0	*0.0035*	*0.0174*	*0.0029*	*0.0312*	*0.1123*	*0.0111*	*0.0111*	*0.0466*	*0.0580*	*0.0386*	*0.0138*	*0.0142*
Urea	-0.9274	0.9676	0.9867	0.9795	0	*0.0145*	*0.0058*	*0.0070*	*0.0876*	*0.0006*	*0.0006*	*0.0196*	*0.0350*	*0.0081*	*0.0028*	*0.0032*
Creat	-0.9937	0.9530	0.9800	0.9403	0.9471	0	*0.0023*	*0.0369*	*0.0129*	*0.0115*	*0.0115*	*0.0069*	*0.0046*	*0.0504*	*0.0042*	*0.0039*
BUN	-0.9607	0.9413	0.9970	0.9820	0.9713	0.9847	0	*0.0368*	*0.0450*	*0.0080*	*0.0080*	*0.0223*	*0.0234*	*0.0467*	*0.0046*	*0.0043*
BCR	0.9058	-0.9568	-0.9294	-0.9114	-0.9674	-0.9009	-0.9010	0	*0.1052*	*0.0063*	*0.0063*	*0.0129*	*0.0327*	*0.0004*	*0.0137*	*0.0156*
Eslen	-0.9693	0.8986	0.8748	0.7894	0.8222	0.9511	0.8867	-0.7985	0	*0.06216*	*0.06216*	*0.0159*	*0.00526*	*0.12693*	*0.0346*	*0.0328*
RC	0.9425	-0.9897	-0.9807	-0.9556	-0.9936	-0.9548	-0.9645	0.9697	-0.8591	0	*1.20E-15*	*0.0108*	*0.02322*	*0.00628*	*0.0005*	*0.0007*
IRRC	-0.9425	0.9897	0.9807	0.9556	0.9936	0.9548	0.9645	-0.9697	0.8591	-1	0	*0.0108*	*0.0232*	*0.0063*	*0.0005*	*0.0007*
PEst	0.9844	-0.9723	-0.9397	-0.8840	-0.9352	-0.9678	-0.9294	0.9511	-0.9435	0.9565	-0.9565	0	*0.0008*	*0.0208*	*0.0066*	*0.0070*
Est	0.9938	-0.9479	-0.9284	-0.8656	-0.9043	-0.9755	-0.9271	0.9086	-0.9731	0.9274	-0.9274	0.9922	0	*0.0478*	*0.0127*	*0.0126*
Mest	-0.8794	0.9590	0.9165	0.8978	0.9640	0.8777	0.8839	-0.9953	0.7710	-0.9698	0.9698	-0.9327	-0.8820	0	*0.0154*	*0.0175*
Dest	-0.9668	0.9924	0.9851	0.9487	0.9826	0.9768	0.9756	-0.9491	0.9050	-0.9944	0.9944	-0.9687	-0.9517	0.9448	0	1.44E-06
DEI	-0.9676	0.9917	0.9852	0.9478	0.9806	0.9781	0.9766	-0.9443	0.9083	-0.9930	0.9930	-0.9674	-0.9517	0.9399	0.9999	0

Correlation (r) value is shown in regular font while P-value is shown in italics font. Statistically significant values between variables are shown as highlighted in regular font ($P < 0.05$).

Abbreviations: **STP:** Serum total protein; **ALT:** Serum alanine transaminase; **AST:** Aspartate aminotransferase; **AAR:** ALT/AST ratio; **Creat:** Creatinine; **BUN:** Blood urea nitrogen; **BCR:** BUN/Creat ratio; **Eslen:** Estrous cycle length; **RC:** Regular cycle; **IRRC:** Irregular cycle; **PEst:** Proestrous; **Est:** Estrous; **Mest:** Metestrous; **Dest:** Diestrous; **DEI:** Diestrous index.

Table S9

One way Analysis of Variance (ANOVA) showing the interaction among lipid profiles and alterations in the estrous cyclicity upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	TC	TAG	HDL	LDL	Eslen	RC	IRRC	PEst	Est	Mest	Dest	DEI
TC	0	*0.999*	*1*	*0.8107*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*
TAG	1.251	0	*0.9988*	*0.2655*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*
HDL	0.0249	1.276	0	*0.8199*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*
LDL	2.544	3.795	2.519	0	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*
Eslen	15.62	16.87	15.6	13.08	0	*1*	*1*	*1*	*1*	*1*	*0.9997*	*0.9978*
RC	16.15	17.4	16.12	13.6	0.5244	0	*1*	*1*	*1*	*1*	*0.9908*	*0.9693*
IRRC	15.84	17.09	15.82	13.3	0.2195	0.3049	0	*1*	*1*	*1*	*0.9985*	*0.992*
PEst	16.1	17.35	16.07	13.55	0.4756	0.04878	0.2561	0	*1*	*1*	*0.9928*	*0.9746*
Est	15.97	17.22	15.95	13.43	0.3481	0.1763	0.1286	0.1275	0	*1*	*0.9965*	*0.9852*
Mest	16.17	17.42	16.14	13.63	0.5467	0.02236	0.3272	0.07114	0.1987	0	*0.9897*	*0.9666*
Mest	14.53	15.78	14.5	11.99	1.093	1.617	1.312	1.569	1.441	1.64	0	*1*
DEI	14.25	15.5	14.23	11.71	1.369	1.894	1.589	1.845	1.717	1.916	0.2764	0

Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons.

F value is shown in regular font while *P*-value is shown in italics font. Statistically significant values between variables are shown as highlighted in italics ($P < 0.05$).

One way ANOVA: $F_{11,48} = 57.43$ and $P = 8.662E\text{-}24$.

Levene's test for homogeneity of variance: $P = 1.829E\text{-}07$.

Welch F test: $F = 41.30$ and $P = 8.381E\text{-}11$.

Abbreviations: **TC:** Total cholesterol; **TAG:** Triacylglycerides; **HDL:** High density lipoprotein; **LDL:** Low density lipoprotein; **Eslen:** Estrous cycle length; **RC:** Regular cycle; **IRRC:** Irregular cycle; **PEst:** Proestrous; **Est:** Estrous; **Mest:** Metestrous; **Dest:** Diestrous; **DEI:** Diestrous index.

Table S10

Linear correlation analysis showing the interaction among lipid profiles and alterations in the estrous cyclicity upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	TC	TAG	HDL	LDL	Eslen	RC	IRRC	PEst	Est	Mest	Dest	DEI
TC	0	*0.00663*	*0.00975*	*0.00327*	*0.08439*	*0.00036*	*0.00036*	*0.01449*	*0.03317*	*0.00202*	*0.0028*	*0.00341*
TAG	0.96866	0	*0.00405*	*0.00064*	*0.02591*	*0.00277*	*0.00277*	*0.00145*	*0.00401*	*0.01558*	*0.00125*	*0.00144*
HDL	-0.9594	-0.9775	0	*0.00117*	*0.07974*	*0.0053*	*0.0053*	*0.01678*	*0.02635*	*0.0156*	*0.00768*	*0.00849*
LDL	0.98046	0.9934	-0.9902	0	*0.05391*	*0.00164*	*0.00164*	*0.00474*	*0.01248*	*0.00654*	*0.00265*	*0.00324*
Eslen	0.8266	0.92186	-0.8331	0.87199	0	*0.06216*	*0.06216*	*0.01597*	*0.00526*	*0.12693*	*0.03464*	*0.03287*
RC	-0.9955	-0.9825	0.97302	-0.9877	-0.8591	0	*1.20E-15*	*0.0108*	*0.02322*	*0.00628*	*0.0005*	*0.00071*
IRRC	0.9955	0.9825	-0.973	0.98766	0.85905	-1	0	*0.0108*	*0.02322*	*0.00628*	*0.0005*	*0.00071*
PEst	-0.9471	-0.9887	0.94162	-0.975	-0.9435	0.95654	-0.9565	0	*0.00082*	*0.02075*	*0.00661*	*0.00702*
Est	-0.9078	-0.9776	0.92097	-0.9521	-0.9731	0.9274	-0.9274	0.99223	0	*0.04779*	*0.01266*	*0.01264*
Mest	0.98581	0.94446	-0.9444	0.96895	0.77101	-0.9698	0.96978	-0.9327	-0.882	0	*0.01545*	*0.01751*
Mest	0.98239	0.98972	-0.9654	0.983	0.905	-0.9944	0.99441	-0.9687	-0.9517	0.94479	0	*1.44E-06*
DEI	0.97992	0.98872	-0.963	0.98057	0.9083	-0.993	0.99298	-0.9674	-0.9517	0.93993	0.99989	0

Correlation (r) value is shown in regular font while P-value is shown in italics font. Statistically significant values between variables are shown as highlighted in regular font ($P < 0.05$).

Abbreviations: **TC:** Total cholesterol; **TAG:** Triacylglycerides; **HDL:** High density lipoprotein; **LDL:** Low density lipoprotein; **Eslen:** Estrous cycle length; **RC:** Regular cycle; **IRRC:** Irregular cycle; **PEst:** Proestrous; **Est:** Estrous; **Mest:** Metestrous; **Dest:** Diestrous; **DEI:** Diestrous index.

Table S11

One way Analysis of Variance (ANOVA) showing the interaction among reproductive hormones and alterations in the estrous cyclicity upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	Est	**LH**	**FSH**	**Prog**	**Eslen**	**RC**	**IRRC**	**PEst**	**Est**	**Mest**	**Dest**	**DEI**
Est	0	*0.00013*	*1*	*0.4427*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*
LH	36.74	0	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*	*0.00013*
FSH	0.1029	36.85	0	*0.3969*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Prog	3.357	33.39	3.459	0	*0.3348*	*0.3179*	*0.3276*	*0.3194*	*0.3235*	*0.3172*	*0.3716*	*0.3812*
Eslen	30.25	37	30.93	33.09	0	*1*	*1*	*1*	*1*	*1*	*1*	*1*
RC	20.29	37.04	30.25	23.52	0.0432	0	*1*	*1*	*1*	*1*	*1*	*1*
IRRC	30.27	37.02	20.74	23.62	0.0181	0.0251	0	*1*	*1*	*1*	*1*	*1*
PEst	30.29	37.04	30.85	33.64	0.0392	0.004	0.0211	0	*1*	*1*	*1*	*1*
Est	30.28	37.03	30.78	43.63	0.0287	0.0145	0.0106	0.0105	0	*1*	*1*	*1*
Mest	30.29	37.04	30.44	43.65	0.0450	0.0018	0.027	0.00586	0.01636	0	*1*	*1*
Dest	30.16	36.91	30.93	23.59	0.0900	0.1332	0.1081	0.1292	0.1187	0.1351	0	*1*
DEI	0.1394	36.88	30.65	43.96	0.1128	0.156	0.1309	0.152	0.1415	0.1578	0.0228	0

Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons.

F value is shown in regular font while *P*-value is shown in italics font. Statistically significant values between variables are shown as highlighted in italics ($P < 0.05$).

One way ANOVA: $F_{11,48} = 112.90$ and $P = 2.136E-30$.

Levene's test for homogeneity of variance: $P = 3.761E-14$.

Welch F test: $F = 47.95$ and $P = 2.168E-11$.

Abbreviations: **Est:** Estradiol; **LH:** Luteinizing hormone; **FSH:** Follicle stimulating hormone; **Prog:** Progesterone; **Eslen:** Estrous cycle length; **RC:** Regular cycle; **IRRC:** Irregular cycle; **PEst:** Proestrous; **Est:** Estrous; **Mest:** Metestrous; **Dest:** Diestrous; **DEI:** Diestrous index.

Table S12

Linear correlation analysis showing the interaction among reproductive hormones and alterations in the estrous cyclicity upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	Est	**LH**	**FSH**	**Prog**	**Eslen**	**RC**	**IRRC**	**PEst**	**Est**	**Mest**	**Dest**	**DEI**
Est	0	*0.03332*	*0.0488*	*0.0802*	*0.1248*	*0.0919*	*0.0919*	*0.08205*	*0.07159*	*0.1388*	*0.0856*	*0.086518*
LH	-0.907	0	*0.00161*	*0.00188*	*0.02436*	*0.01087*	*0.01087*	*0.01403*	*0.01179*	*0.05339*	*0.00471*	*0.00432*
FSH	-0.88	0.98785	0	*0.0017*	*0.0445*	*0.0014*	*0.0014*	*0.011*	*0.01648*	*0.0206*	*0.0007*	*0.000762*
Prog	-0.833	0.98647	0.9873	0	*0.0179*	*0.0044*	*0.00442*	*0.00761*	*0.00873*	*0.0357*	*0.0006*	*0.000447*
Eslen	0.7737	-0.925	-0.8875	-0.939	0	*0.0622*	*0.06216*	*0.01597*	*0.00526*	*0.1269*	*0.0346*	*0.032869*
RC	-0.816	0.95637	0.9889	0.9761	-0.8591	0	*1.20E-15*	*0.0108*	*0.02322*	*0.0063*	*0.0005*	*0.000706*
IRRC	0.8163	-0.9564	-0.9889	-0.976	0.8591	-1	0	*0.0108*	*0.02322*	*0.0063*	*0.0005*	*0.000706*
PEst	-0.83	0.94824	0.956	0.9656	-0.9435	0.9565	-0.95654	0	*0.00082*	*0.0208*	*0.0066*	*0.007024*
Est	-0.845	0.95392	0.9423	0.9623	-0.9731	0.9274	-0.9274	0.99223	0	*0.0478*	*0.0127*	*0.012643*
Mest	0.7566	-0.8728	-0.9331	-0.903	0.7710	-0.97	0.96978	-0.9327	-0.882	0	*0.0154*	*0.017513*
Dest	0.8249	-0.9751	-0.9929	-0.993	0.9050	-0.994	0.99441	-0.9687	-0.9517	0.9448	0	*1.44E-06*
DEI	0.8236	-0.9765	-0.9926	-0.995	0.9083	-0.993	0.99298	-0.9674	-0.9517	0.9399	0.9999	0

Correlation (r) value is shown in regular font while P-value is shown in italics font. Statistically significant values between variables are shown as highlighted in regular font ($P < 0.05$).

Abbreviations: **Est:** Estradiol; **LH:** Luteinizing hormone; **FSH:** Follicle stimulating hormone; **Prog:** Progesterone; **Eslen:** Estrous cycle length; **RC:** Regular cycle; **IRRC:** Irregular cycle; **PEst:** Proestrous; **Est:** Estrous; **Mest:** Metestrous; **Dest:** Diestrous; **DEI:** Diestrous index.

Table S13

Linear correlation analysis showing the interaction among lipid peroxidation and antioxidant enzyme parameters and alterations in the estrous cyclicity upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	MDA	CAT	SOD	GST	GSH	Eslen	RC	IRRC	PEst	Est	Mest	Dest	DEI
MDA	0	*0.0056*	*0.0490*	*0.0112*	*0.0662*	*0.0332*	*0.0160*	*0.0160*	*0.0339*	*0.0307*	*0.0784*	*0.0073*	*0.0063*
CAT	-0.9720	0	*0.0101*	*0.0005*	*0.0144*	*0.0253*	*0.0019*	*0.0019*	*0.0047*	*0.0074*	*0.0222*	*0.0003*	*0.0002*
SOD	-0.8800	0.9584	0	*0.0140*	*0.0135*	*0.0940*	*0.0055*	*0.0055*	*0.0129*	*0.0274*	*0.0044*	*0.0108*	*0.0123*
GST	-0.9555	0.9942	0.9483	0	*0.0066*	*0.0205*	*0.0022*	*0.0022*	*0.0023*	*0.0056*	*0.0181*	*0.0003*	*0.0003*
GSH	-0.8529	0.9473	0.9495	0.9686	0	*0.0438*	*0.0118*	*0.0118*	*0.0022*	*0.0101*	*0.0079*	*0.0123*	*0.0134*
Eslen	0.9077	-0.9231	-0.8135	-0.9332	-0.8887	0	*0.0622*	*0.0622*	*0.0160*	*0.0053*	*0.1269*	*0.0346*	*0.0329*
RC	-0.9436	0.9862	0.9723	0.9849	0.9538	-0.8591	0	*1.20E-15*	*0.0108*	*0.0232*	*0.0063*	*0.0005*	*0.0007*
IRRC	0.9436	-0.9862	-0.9723	-0.9849	-0.9538	0.8591	-1	0	*0.0108*	*0.0232*	*0.0063*	*0.0005*	*0.0007*
PEst	-0.9064	0.9751	0.9511	0.9844	0.9850	-0.9435	0.9565	-0.9565	0	*0.0008*	*0.0208*	*0.0066*	*0.0070*
Est	-0.9125	0.9663	0.9190	0.9721	0.9584	-0.9731	0.9274	-0.9274	0.9922	0	*0.0478*	*0.0127*	*0.0126*
Mest	0.8351	-0.9296	-0.9763	-0.9387	-0.9647	0.7710	-0.9698	0.9698	-0.9327	-0.8820	0	*0.0154*	*0.0175*
Dest	0.9665	-0.9965	-0.9566	-0.9958	-0.9527	0.9050	-0.9944	0.9944	-0.9687	-0.9517	0.9448	0	*1.44E-06*
DEI	0.9697	-0.9966	-0.9525	-0.9957	-0.9497	0.9083	-0.9930	0.9930	-0.9674	-0.9517	0.9399	0.9999	0

Correlation (r) value is shown in regular font while P-value is shown in italics font. Statistically significant values between variables are shown as highlighted in regular font ($P < 0.05$).

Abbreviations: **MDA:** Malondialdehyde; **CAT:** Catalase; **SOD:** Super oxide dismutase; **GST:** Glutathione S transferase; **GSH:** Reduced glutathione; **Eslen:** Estrous cycle length; **RC:** Regular cycle; **IRRC:** Irregular cycle; **PEst:** Proestrous; **Est:** Estrous; **Mest:** Metestrous; **Dest:** Diestrous; **DEI:** Diestrous index.

Table S14

Ovarian biopsy score (OBS) count method.

OBS score	Oogenesis events
Score 10	Complete oogenesis with abundant ovarian follicles
Score 9	Many primordial and primary and with irregular germinal epithelium
Score 8	Less number of secondary follicles and Corpus luteum
Score 7	No mature Graafian follicles, but many primordial follicles
Score 6	Few primordial follicles
Score 5	Few Graafian follicles with few atretic follicles
Score 4	Many atretic follicles with few Graafian follicles
Score 3	Absence of corpus luteum
Score 2	Presence of many atretic follicles
Score 1	Vacuolated ovarian cortex and medulla with complete oogenesis arrest

Table S15

Long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) on histo-morphometric and immuno-histochemical alterations in the ovary of female mice

Parameter	Experimental group						
Follicle type	Control	Diosgenin 10 mg/kg	Diosgenin 50 mg/kg	Diosgenin 100 mg/kg	Diosgenin 200 mg/kg	$F_{4,49}$ value	*p* value
No. of follicles type/ovary							
Primordial follicle	6.18 ± 0.22a	4.95 ± 0.35bc	4.58 ± 0.12b	4.16 ± 0.11b	4.07 ± 0.15bc	16.582	0.0001
Primary follicle	12.17 ± 0.68a	11.47 ± 0.89ab	10.32 ± 0.51abd	9.24 ± 0.16bc	8.12 ± 0.14cd	8.690	0.0001
Secondary Follicle	17.05 ± 0.32a	16.75 ± 0.72ab	15.27 ± 0.15bc	15.01 ± 0.11c	12.44 ± 0.40d	20.587	0.0001
Tertiary follicle	25.18 ± 0.95a	24.71 ± 0.82a	23.89 ± 0.22a	23.12 ± 0.85a	20.14 ± 0.35b	8.017	0.0001
Atretic follicle	20.26 ± 0.36a	22.54 ± 0.24b	22.98 ± 0.10b	24.08 ± 0.45bc	25.18 ± 0.59c	22.797	0.0001
Corpus Luteum	28.07 ± 0.62a	27.64 ± 0.18a	26.03 ± 0.19bc	25.78 ± 0.25cd	24.46 ± 0.26d	18.463	0.0001
Diameter of follicle type							
Mean primordial follicle diameter (MPmFD, µm)	47.31 ± 1.67a	52.33 ± 2.54a	59.12 ± 1.09a	66.43 ± 2.76b	78.66 ± 4.17b	21.420	0.0001
Mean primary follicle diameter (MPFD, µm)	71.83 ± 1.87a	74.65 ± 2.26a	81.44 ± 2.87a	90.72 ± 1.77b	94.77 ± 1.99b	20.079	0.0001
Mean secondary follicle diameter (MSFD, µm)	105.1 ± 2.81a	145.82 ± 2.38b	178.94 ± 1.86c	202.99 ± 2.41d	212.46 ± 3.18d	208.87	0.0001
Mean tertiary follicle diameter (MGFD, µm)	225.7 ± 3.41a	244.80 ± 3.87a	367.41 ± 2.08b	519.03 ± 2.83c	565.27 ± 3.66d	265.88	0.0001
Ratio of oocyte in the secondary follicle to granulosa cell layer							
Ratio of oocyte to granulosa cell layer in the secondary follicles	32.71 ± 1.05a	23.84 ± 1.63b	20.28 ± 1.42bc	19.75 ± 1.26bc	16.68 ± 1.55c	19.492	0.0001
Ovary lesion score							
Ovarian tissue damage score (OTDS)	0a	1.15 ± 0.21a	3.07 ± 0.77b	3.36 ± 0.45b	3.55 ± 1.08b	106.40	0.0001
Ovarian biopsy score (OBS)	9.78 ± 0.77a	7.12 ± 0.45b	6.15 ± 0.83b	4.59 ± 0.22c	3.76 ± 0.77c	13.043	0.0001
Immunoreactivity ratio							
PCNA immunoreactivity ratio	78.54 ± 1.32a	66.82 ± 3.66a	50.34 ± 2.88b	44.71 ± 4.91b	25.87 ± 1.65c	41.153	0.0001
Visfatin immunoreactivity ratio	46.45 ± 1.24a	37.87 ± 2.66b	24.79 ± 1.99c	18.45 ± 1.02d	15.09 ± 0.49d	63.574	0.0001

Values are expressed as mean ± standard error mean (n = (10 random non-overlapping fields/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $p < 0.05$ and with similar letters indicate that treatment groups are not statistically significant at $p > 0.05$.

Ovarian tissue damage score (OTDS): Normal: score - 0; < 25% damage: score 1; 25 - 50% damage: score 2; 50 - 75% damage: score 3; 75 - 100% damage: score 4.

PCNA/Visfatin immunoreactivity ratio = Positively reacted cells/total number of cells×100

Table S16

Linear correlation analysis showing the interaction among liver and kidney function parameters and alterations in the ovarian follicles number and size, ovary damage score and ovary biopsy score upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	STP	ALT	AST	Urea	Creat	BUN	Primfol	Prifl	secfl	Tertfl	Artfl	CL	MPmFD	MPFD	MSFD	MGFD	OvDS	OBS
STP	0	*0.0126*	*0.0102*	*0.0232*	*0.0006*	*0.0093*	*0.0559*	*0.0039*	*0.0025*	*0.00228*	*0.6121*	*0.0014*	*0.0002*	*0.01075*	*0.038*	*0.0178*	*0.738*	*0.0276*
ALT	-0.9518	0	*0.0104*	*0.007*	*0.0122*	*0.0169*	*0.155*	*0.0031*	*0.0325*	*0.03663*	*0.3978*	*0.0203*	*0.0065*	*0.00998*	*0.0102*	*0.0134*	*0.6781*	*0.0162*
AST	-0.9581	0.9575	0	*0.0018*	*0.0034*	*0.0002*	*0.1861*	*0.0008*	*0.0349*	*0.03541*	*0.4356*	*0.0327*	*0.0043*	*1.87E-06*	*0.012*	*0.0002*	*0.4351*	*0.013*
Urea	-0.9274	0.9676	0.9867	0	*0.0145*	*0.0058*	*0.2598*	*0.0025*	*0.0662*	*0.06764*	*0.2936*	*0.0529*	*0.0119*	*0.00191*	*0.0016*	*0.003*	*0.4164*	*0.0042*
Creat	-0.9937	0.9530	0.9800	0.9471	0	*0.0023*	*0.0824*	*0.0016*	*0.006*	*0.00601*	*0.5978*	*0.0065*	*0.0002*	*0.00352*	*0.0328*	*0.0066*	*0.6223*	*0.0267*
BUN	-0.9607	0.9413	0.9970	0.9713	0.9847	0	*0.1651*	*0.0019*	*0.0286*	*0.02875*	*0.509*	*0.0308*	*0.0044*	*0.0002*	*0.0231*	*0.0004*	*0.4375*	*0.0226*
Primfol	0.8688	-0.7375	-0.7023	-0.6248	-0.8294	-0.7257	0	*0.1413*	*0.0181*	*0.01789*	*0.8273*	*0.0252*	*0.0819*	*0.18689*	*0.3049*	*0.212*	*0.7063*	*0.2597*
Prifl	0.9781	-0.9810	-0.9927	-0.9838	-0.9877	-0.9863	0.7536	0	*0.0208*	*0.02168*	*0.4409*	*0.0157*	*0.0009*	*0.0008*	*0.0095*	*0.0025*	*0.5546*	*0.0096*
secfl	0.9838	-0.9089	-0.9046	-0.8529	-0.9707	-0.9166	0.9385	0.9326	0	*2.53E-05*	*0.818*	*0.0006*	*0.0062*	*0.03511*	*0.0962*	*0.045*	*0.9004*	*0.0791*
Tertfl	0.9846	-0.9014	-0.9036	-0.8508	-0.9707	-0.9162	0.9391	0.9307	0.9992	0	*0.8157*	*0.0007*	*0.0064*	*0.03597*	*0.0968*	*0.0469*	*0.8919*	*0.0765*
Artfl	-0.3097	0.4939	0.4601	0.5914	0.3215	0.3962	0.1361	-0.4554	-0.1434	-0.1453	0	*0.7111*	*0.5497*	*0.44012*	*0.2013*	*0.4513*	*0.3736*	*0.2299*
CL	0.9888	-0.9336	-0.9086	-0.8735	-0.9692	-0.9122	0.9234	0.9442	0.9935	0.9928	-0.2289	0	*0.0042*	*0.03327*	*0.0695*	*0.0455*	*0.9236*	*0.0547*
MPmFD	-0.9965	0.9692	0.9766	0.9535	0.9969	0.9761	-0.8302	-0.9918	-0.9702	-0.9695	0.3617	-0.9770	0	*0.00448*	*0.0242*	*0.0087*	*0.6668*	*0.0192*
MPFD	-0.9567	0.9588	0.9999	0.9864	0.9795	0.9970	-0.7014	-0.9924	-0.9042	-0.9026	0.4561	-0.9076	0.9759	0	*0.0126*	*0.0001*	*0.4357*	*0.0144*
MSFD	-0.8989	0.9583	0.9534	0.9880	0.9084	0.9277	-0.5804	-0.9601	-0.8105	-0.8096	0.6857	-0.8480	0.9254	0.9518	0	*0.0173*	*0.4596*	*0.001*
MGFD	-0.9393	0.9498	0.9965	0.9816	0.9689	0.9949	-0.6742	-0.9835	-0.8866	-0.8834	0.4462	-0.8858	0.9624	0.9976	0.9403	0	*0.3981*	*0.0226*
OvDS	-0.2073	0.2556	0.4605	0.4772	0.3013	0.4584	0.2328	-0.3576	-0.0783	-0.0850	0.5159	-0.0601	0.2649	0.4600	0.4389	0.4936	0	*0.4937*
OBS	0.9185	-0.9429	-0.9507	-0.9769	-0.9203	-0.9287	0.6249	0.9598	0.8341	0.8378	-0.6554	0.8708	-0.9361	-0.9473	-0.9914	-0.9288	-0.4094	0

Correlation (r) value is shown in regular font while P-value is shown in italics font. Statistically significant values between variables are shown as highlighted in regular font ($P < 0.05$).

Abbreviations: **STP:** Serum total protein; **ALT:** Serum alanine transaminase; **AST:** Aspartate aminotransferase; **Creat:** Creatinine; **BUN:** Blood urea nitrogen; **Primfol:** No. of Primordial follicle; **Prifl:** No. of primary follicle; **Secfl:** No. of secondary follicle; **Tertfl:** No. of tertiary follicle; **Artfl:** No. of atretic follicle; **CL:** No. of corpus luteum; **MPmFD:** Mean primordial follicle diameter; **MPFD:** Mean primary follicle diameter; **MSFD:** Mean secondary follicle diameter; **MGFD:** Mean tertiary follicle diameter; **OvDS:** Ovary damage score; **OBS:** Ovary biopsy score.

Table S17

One way Analysis of Variance (ANOVA) showing the interaction among reproductive hormones and alterations in the ovarian follicles number and size, ovary damage score and ovary biopsy score upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	Est	**LH**	**FSH**	**Prog**	**Primfol**	**Prifl**	**Secfl**	**Tertfl**	**Artfl**	**CL**	**MPmFD**	**MPFD**	**MSFD**	**MGFD**	**OvDS**	**OBS**
Est	0	*0.0001*	*1*	*0.587*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0003*	*0.0001*	*0.0001*
LH	36.44	0	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*
FSH	0.10	36.54	0	*0.536*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0002*	*0.0001*	*0.0001*
Prog	3.33	3.11	3.43	0	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Primfol	32.24	36.68	30.14	23.57	0	*1*	*1*	*1*	*1*	*1*	*1*	*0.9989*	*0.5602*	*0.0002*	*0.0001*	*0.0001*
Prifl	30.15	36.59	20.05	23.48	0.09	0	*1*	*1*	*1*	*1*	*1*	*0.9994*	*0.6039*	*0.0002*	*0.0001*	*0.0001*
Secfl	30.04	36.48	30.06	23.37	0.20	0.11	0	*1*	*1*	*1*	*1*	*0.9998*	*0.6575*	*0.0002*	*0.0001*	*0.0001*
Tertfl	20.12	36.32	30.23	33.21	0.36	0.27	0.17	0	*1*	*1*	*1*	*0.9999*	*0.7353*	*0.0003*	*0.0001*	*0.0001*
Artfl	24.08	36.36	30.18	33.25	0.32	0.23	0.12	0.04	0	*1*	*1*	*0.9999*	*0.7158*	*0.0003*	*0.0001*	*0.0001*
CL	22.11	36.33	30.21	33.22	0.35	0.26	0.15	0.01	0.03	0	*1*	*0.9999*	*0.729*	*0.0003*	*0.0001*	*0.0001*
MPmFD	25.90	35.54	31.00	32.43	1.14	1.05	0.94	0.77	0.82	0.79	0	*1*	*0.9647*	*0.0013*	*0.0001*	*0.0001*
MPFD	21.35	35.09	31.45	31.98	1.59	1.50	1.39	1.23	1.27	1.24	0.45	0	*0.9958*	*0.0037*	*0.0001*	*0.0001*
MSFD	23.15	33.3	33.25	30.18	3.38	3.30	3.19	3.02	3.06	3.04	2.25	1.79	0	*0.1392*	*0.0001*	*0.0001*
MGFD	27.61	28.83	37.72	34.29	7.85	7.77	7.66	7.49	7.53	7.51	6.72	6.26	4.47	0	*0.0019*	*0.00*
OvDS	21.07	35.37	31.17	32.26	31.31	21.22	21.11	30.95	30.99	30.96	10.17	10.28	12.07	16.54	0	*1.00*
OBS	20.23	36.67	30.13	33.56	30.00	20.08	20.19	30.36	30.31	30.34	11.13	11.59	13.38	17.85	1.31	0

Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons.

F value is shown in regular font while *P*-value is shown in italics font. Statistically significant values between variables are shown as highlighted in italics ($P < 0.05$).

One way ANOVA: $F_{15,64} = 82.18$ and $P = 7.401E\text{-}36$.

Levene's test for homogeneity of variance: $P = 4.18E\text{-}15$.

Welch F test: $F = 166.20$ and $P = 5.915E\text{-}20$.

Abbreviations: **Est:** Estradiol; **LH:** Luteinizing hormone; **FSH:** Follicle stimulating hormone; **Prog:** Progesterone; **Primfol:** No. of Primordial follicle; **Prifl:** No. of primary follicle; **Secfl:** No. of secondary follicle; **Tertfl:** No. of tertiary follicle; **Artfl:** No. of atretic follicle; **CL:** No. of corpus luteum; **MPmFD:** Mean primordial follicle diameter; **MPFD:** Mean primary follicle diameter; **MSFD:** Mean secondary follicle diameter; **MGFD:** Mean tertiary follicle diameter; **OvDS:** Ovary damage score; **OBS:** Ovary biopsy score.

Table S18

Linear correlation analysis showing the interaction among reproductive hormones and alterations in the ovarian follicles number and size, ovary damage score and ovary biopsy score upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	Est	**LH**	**FSH**	**Prog**	**Primfol**	**Prifl**	**Secfl**	**Tertfl**	**Artfl**	**CL**	**MPmFD**	**MPFD**	**MSFD**	**MGFD**	**OvDS**	**OBS**
Est	0	*0.0333*	*0.0488*	*0.0802*	*0.2221*	*0.0547*	*0.0870*	*0.0756*	*0.5551*	*0.0927*	*0.0518*	*0.0442*	*0.0877*	*0.0548*	*0.3884*	*0.0542*
LH	-0.9075	0	*0.0016*	*0.0019*	*0.1136*	*0.0017*	*0.0133*	*0.0132*	*0.5753*	*0.0155*	*0.0014*	*0.0013*	*0.0307*	*0.0027*	*0.5291*	*0.0265*
FSH	-0.8803	0.9879	0	*0.0017*	*0.1717*	*0.0001*	*0.0299*	*0.0308*	*0.4219*	*0.0254*	*0.0025*	*0.0001*	*0.0087*	*0.0008*	*0.4836*	*0.0098*
Prog	-0.8325	0.9865	0.9873	0	*0.1071*	*0.0008*	*0.0119*	*0.0139*	*0.5478*	*0.0106*	*0.0010*	*0.0027*	*0.0245*	*0.0040*	*0.6251*	*0.0274*
Primfol	-0.6635	0.7877	0.7183	0.7961	0	*0.1413*	*0.0181*	*0.0179*	*0.8273*	*0.0252*	*0.0819*	*0.1869*	*0.3049*	*0.2120*	*0.7063*	*0.2597*
Prifl	-0.8708	0.9873	0.9978	0.9922	0.7536	0	*0.0208*	*0.02168*	*0.4409*	*0.0157*	*0.0009*	*0.0008*	*0.0095*	*0.0025*	*0.5546*	*0.0096*
Secfl	-0.8230	0.9500	0.9139	0.9537	0.9385	0.9326	0	*2.53E-05*	*0.8180*	*0.0006*	*0.0062*	*0.0351*	*0.0962*	*0.0450*	*0.9004*	*0.0791*
Tertfl	-0.8391	0.9502	0.9122	0.9485	0.9391	0.9307	0.9992	0	*0.816*	*0.0007*	*0.0064*	*0.0360*	*0.0968*	*0.0469*	*0.8919*	*0.0765*
Artfl	0.9572	-0.9403	-0.9722	-0.9633	0.1361	-0.4554	-0.1434	-0.1453	0	*0.7111*	*0.5497*	*0.4401*	*0.2013*	*0.4513*	*0.3736*	*0.2299*
CL	-0.8151	0.9446	0.9229	0.9570	0.9234	0.9442	0.9935	0.9928	-0.229	0	*0.0042*	*0.0333*	*0.0695*	*0.0455*	*0.9236*	*0.0547*
MPmFD	0.8754	-0.9889	-0.9835	-0.9914	-0.8302	-0.9918	-0.9702	-0.9695	0.362	-0.9770	0	*0.0045*	*0.0242*	*0.0087*	*0.6668*	*0.0192*
MPFD	0.8881	-0.9893	-0.9981	-0.9828	-0.7014	-0.9924	-0.9042	-0.9026	0.456	-0.9076	0.9759	0	*0.0126*	*0.0001*	*0.4357*	*0.0144*
MSFD	0.8220	-0.9123	-0.9624	-0.9247	-0.5804	-0.9601	-0.8105	-0.8096	0.686	-0.8480	0.9254	0.9518	0	*0.0173*	*0.4596*	*0.0010*
MGFD	0.8706	-0.9830	-0.9925	-0.9775	-0.6742	-0.9835	-0.8866	-0.8834	0.8446	-0.8858	0.9624	0.9976	0.9403	0	*0.3981*	*0.0226*
OvDS	0.5024	-0.9791	-0.4181	-0.6989	0.9328	-0.9576	-0.9783	-0.0850	0.516	-0.8601	0.8649	0.8600	0.8389	0.8936	0	*0.4937*
OBS	-0.8716	0.9206	0.9592	0.9189	0.6249	0.9598	0.8341	0.8378	-0.655	0.8708	-0.9361	-0.9473	-0.9914	-0.9288	-0.4094	0

Correlation (r) value is shown in regular font while P-value is shown in italics font. Statistically significant values between variables are shown as highlighted in regular font ($P < 0.05$). Abbreviations: **Est:** Estradiol; **LH:** Luteinizing hormone; **FSH:** Follicle stimulating hormone; **Prog:** Progesterone; **Primfol:** No. of Primordial follicle; **Prifl:** No. of primary follicle; **Secfl:** No. of secondary follicle; **Tertfl:** No. of tertiary follicle; **Artfl:** No. of atretic follicle; **CL:** No. of corpus luteum; **MPmFD:** Mean primordial follicle diameter; **MPFD:** Mean primary follicle diameter; **MSFD:** Mean secondary follicle diameter; **MGFD:** Mean tertiary follicle diameter; **OvDS:** Ovary damage score; **OBS:** Ovary biopsy score.

Table S19

Linear correlation analysis showing the interaction among lipid peroxidation and antioxidant enzyme parameters and alterations in the ovarian follicles number and size, ovary damage score and ovary biopsy score upon long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) in the female Swiss albino mice.

	MDA	CAT	SOD	GST	GSH	Primfol	Prifl	Secfl	Tertfl	Artfl	CL	MPmFD	MPFD	MSFD	MGFD	OvDS	OBS
MDA	0	*0.0056*	*0.0490*	*0.0112*	*0.0662*	*0.1376*	*0.0068*	*0.0231*	*0.0259*	*0.6421*	*0.0307*	*0.0082*	*0.0037*	*0.0505*	*0.0025*	*0.5090*	*0.0573*
CAT	-0.9720	0	*0.0101*	*0.0005*	*0.0144*	*0.1257*	*1.89E-05*	*0.0162*	*0.0173*	*0.4719*	*0.0123*	*0.0005*	*0.0012*	*0.0128*	*0.0031*	*0.5840*	*0.0130*
SOD	-0.8800	0.9584	0	*0.0140*	*0.0135*	*0.2465*	*0.0072*	*0.0708*	*0.0685*	*0.2481*	*0.0492*	*0.0156*	*0.0111*	*0.0010*	*0.0183*	*0.4895*	*0.0000*
GST	-0.9555	0.9942	0.9483	0	*0.0066*	*0.1122*	*0.0009*	*0.0153*	*0.0174*	*0.4711*	*0.0090*	*0.0012*	*0.0058*	*0.0147*	*0.0093*	*0.6839*	*0.0166*
GSH	-0.8529	0.9473	0.9495	0.9686	0	*0.1363*	*0.0145*	*0.0397*	*0.0414*	*0.3581*	*0.0185*	*0.0147*	*0.0356*	*0.0151*	*0.0494*	*0.8370*	*0.0135*
Primfol	-0.7580	0.7725	0.6383	0.7896	0.7595	0	*0.1413*	*0.0181*	*0.0179*	*0.8273*	*0.0252*	*0.0819*	*0.1869*	*0.3049*	*0.2120*	*0.7063*	*0.2597*
Prifl	-0.9682	0.9994	0.9667	0.9918	0.9470	0.7536	0	*0.0208*	*0.0217*	*0.4409*	*0.0157*	*0.0009*	*0.0008*	*0.0095*	*0.0025*	*0.5546*	*0.0096*
Secfl	-0.9276	0.9429	0.8460	0.9452	0.8958	0.9385	0.9326	0	*2.53E-05*	*0.8180*	*0.0006*	*0.0062*	*0.0351*	*0.0962*	*0.0450*	*0.9004*	*0.0791*
Tertfl	-0.9220	0.9403	0.8495	0.9402	0.8928	0.9391	0.9307	0.9992	0	*0.8157*	*0.0007*	*0.0064*	*0.0360*	*0.0968*	*0.0469*	*0.8919*	*0.0765*
Artfl	0.2850	-0.4282	-0.6367	-0.4289	-0.5302	0.1361	-0.4554	-0.1434	-0.1453	0	*0.7111*	*0.5497*	*0.4401*	*0.2013*	*0.4513*	*0.3736*	*0.2299*
CL	-0.9125	0.9527	0.8797	0.9616	0.9376	0.9234	0.9442	0.9935	0.9928	-0.229	0	*0.0042*	*0.0333*	*0.0695*	*0.0455*	*0.9236*	*0.0547*
MPmFD	0.9640	-0.9945	-0.9445	-0.9897	-0.9467	-0.8302	-0.9918	-0.9702	-0.9695	0.3617	-0.9770	0	*0.0045*	*0.0242*	*0.0087*	*0.6668*	*0.0192*
MPFD	0.9788	-0.9900	-0.9557	-0.9714	-0.9033	-0.7014	-0.9924	-0.9042	-0.9026	0.4561	-0.9076	0.9759	0	*0.0126*	*0.0001*	*0.4357*	*0.0144*
MSFD	0.8775	-0.9512	-0.9909	-0.9467	-0.9457	-0.5804	-0.9601	-0.8105	-0.8096	0.6857	-0.8480	0.9254	0.9518	0	*0.0173*	*0.4596*	*0.0010*
MGFD	0.9835	-0.9813	-0.9381	-0.9607	-0.8793	-0.6742	-0.9835	-0.8866	-0.8834	0.4462	-0.8858	0.9624	0.9976	0.9403	0	*0.3981*	*0.0226*
OvDS	0.3963	-0.3330	-0.4130	-0.2510	-0.1284	0.2328	-0.3576	-0.0783	-0.0850	0.5159	-0.0601	0.2649	0.4600	0.4389	0.4936	0	*0.4937*
OBS	-0.8667	0.9508	0.9996	0.9420	0.9496	0.6249	0.9598	0.8341	0.8378	-0.6554	0.8708	-0.9361	-0.9473	-0.9914	-0.9288	-0.4094	0

Correlation (r) value is shown in regular font while P-value is shown in italics font. Statistically significant values between variables are shown as highlighted in regular font ($P < 0.05$). Abbreviations: **MDA:** Malondialdehyde; **CAT:** Catalase; **SOD:** Super oxide dismutase; **GST:** Glutathione S transferase; **GSH:** Reduced glutathione; **Primfol:** No. of Primordial follicle; **Prifl:** No. of primary follicle; **Secfl:** No. of secondary follicle; **Tertfl:** No. of tertiary follicle; **Artfl:** No. of atretic follicle; **CL:** No. of corpus luteum; **MPmFD:** Mean primordial follicle diameter; **MPFD:** Mean primary follicle diameter; **MSFD:** Mean secondary follicle diameter; **MGFD:** Mean tertiary follicle diameter; **OvDS:** Ovary damage score; **OBS:** Ovary biopsy score.

Table S20

Chronic exposure of diosgenin (repeated dose 90-day oral toxicity study) affects fertility parameters in the Swiss albino mice.

Parameter	Experimental group					
	Control	Diosgenin 10 mg/kg	Diosgenin 50 mg/kg	Diosgenin 100 mg/kg	Diosgenin 200 mg/kg	$F_{4,24}$ value
No. of plug/sperm-positive females (%)	96.78 ± 1.45a	81.65 ± 1.82b	76.41 ± 1.05b	63.28 ± 1.10c	57.39 ± 1.84c	108.88
No. of pregnant females (%)	89.41 ± 1.95a	78.55 ± 1.05b	70.65 ± 1.38c	68.74 ± 1.95c	60.65 ± 1.20d	49.212
Gestational length (day)	22.05 ± 1.38a	21.25 ± 1.88a	20.95 ± 1.71a	20.56 ± 1.52a	20.08 ± 1.92a	0.1920
No. of live pups/litter (PND 0)	9.45 ± 0.71a	9.04 ± 0.45ab	8.65 ± 0.39ab	7.42 ± 0.16b	6.55 ± 0.24c	7.696
No. of live litters on PND 0	21.05 ± 0.36a	19.00 ± 0.12b	18.06 ± 0.28b	17.10 ± 0.11c	17.00 ± 0.20c	50.725
No. of live litters on PND 4	21.00 ± 0.02a	18.35 ± 0.14b	17.30 ± 0.45b	16.09 ± 0.18c	15.51 ± 0.40c	56.785
Female mating index (%)	90.33 ± 1.85a	85.69 ± 1.26ab	80.40 ± 1.20bc	76.51 ± 1.62c	68.30 ± 1.15d	34.627
Male mating index (%)	92.68 ± 1.25a	82.16 ± 1.35b	74.51 ± 1.45c	70.25 ± 1.10cd	65.48 ± 1.30d	68.101
Fecundity index (%)	89.02 ± 0.96a	80.58 ± 1.16bc	76.75 ± 1.19cd	72.58 ± 1.51d	67.25 ± 1.10e	47.342
Gestational index (%)	95.65 ± 1.08a	91.28 ± 1.49a	85.16 ± 1.40b	79.05 ± 1.32c	70.16 ± 1.66d	51.523

Values are expressed as mean ± standard error mean (n = 5/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $P < 0.0001$ and with similar letters indicate that treatment groups are not statistically significant at $P > 0.0001$.

Female mating index = {(No. of plug or sperm-positive females) / (No. of females paired) × 100};.

Male mating index {(No. of males impregnating) / (No. of males paired) × 100}.

Fecundity index {(No: of pregnant females) / (No: of plug or sperm-positive females) × 100}.

Gestational index {(No: of live litters; PND 0) / (No: of total litters; PND 0) × 100}.

Table S21

Endocrine disrupting and reproductive toxicant activity of diosgenin in the F1 generation of male and female Swiss albino mice (F1 extended one-generation reproductive toxicity study).

Parameter	Experimental group – F_0 generation exposed to diosgenin				F value
	Control	Diosgenin 50 mg/kg	Diosgenin 100 mg/kg	Diosgenin 200 mg/kg	
Reproductive hormone levels – F_1 generation Female †					
Serum estradiol (pg/mL)	183.54 ± 5.57a	151.03 ± 2.33b	127.16 ± 0.22c	101.98 ± 0.45d	131.66
Serum luteinizing hormone (LH, pg/mL)	1.54 ± 0.06a	1.31 ± 0.04b	1.06 ± 0.05c	0.78 ± 0.02d	52.786
Serum follicle stimulating hormone (FSH, IU/L)	80.17 ± 1.05a	65.98 ± 3.45b	51.72 ± 1.66c	39.11 ± 0.87d	76.304
Serum progesterone (ng/mL)	275.91 ± 10.88a	220.83 ± 5.76b	178.96 ± 7.83c	125.99 ± 5.19d	67.366
Reproductive hormone levels - F_1 generation Male †					
Serum testosterone (ng/mL)	11.97 ± 0.33a	10.45 ± 0.37b	7.15 ± 0.26c	3.73 ± 0.11d	172.70
Serum luteinizing hormone (LH, pg/mL)	1.35 ± 0.04a	1.06 ± 0.01b	0.87 ± 0.02c	0.63 ± 0.02d	148.20
Serum follicle stimulating hormone (FSH, IU/L)	77.36 ± 1.43a	52.87 ± 1.92b	49.56 ± 0.45b	27.44 ± 1.22c	225.07
Histomorphometric analysis of the number of follicles type/ovary and ovarian damage and biopsy score– F_1 generation Female ††					
Primordial follicle (PrF)	7.22 ± 0.34a	5.36 ± 0.17b	4.05 ± 0.24c	3.67 ± 0.11bc	47.973
Primary follicle (PF)	15.26 ± 0.17a	13.45 ± 0.55b	11.24 ± 0.16c	7.09 ± 0.13d	132.60
Secondary Follicle (SF)	16.01 ± 0.66a	15.17 ± 0.41a	13.86 ± 0.05a	10.01 ± 0.33b	39.385
Tertiary follicle (TF)	21.11 ± 0.11a	20.53 ± 0.53a	19.77 ± 0.21b	15.12 ± 0.17c	331.41
Atretic follicle (A)	17.87 ± 0.35a	20.46 ± 0.26b	23.04 ± 0.23bc	28.74 ± 0.77d	103.40
Corpus Luteum (CL)	25.99 ± 0.11a	22.45 ± 0.12b	17.65 ± 0.33c	14.62 ± 0.38d	363.23
Ovarian tissue damage score (OTDS)	0a	3.78 ± 0.19b	4.15 ± 0.14b	6.11 ± 0.08c	420.02
Ovarian biopsy score (OBS)	9.41 ± 0.15a	7.32 ± 0.13b	5.22 ± 0.04c	2.93 ± 0.21d	363.65
Mean primordial follicle diameter (MPmFD, µm)	57.25 ± 2.05a	68.07 ± 1.96b	74.83 ± 1.11c	82.19 ± 0.65d	46.308
Mean primary follicle diameter (MPFD, µm)	65.08 ± 0.68a	71.98 ± 1.22b	80.05 ± 0.39c	97.44 ± 2.38d	100.19
Mean secondary follicle diameter (MSFD, µm)	112.96 ± 2.65a	133.75 ± 5.94b	178.07 ± 3.95c	204.56 ± 1.34d	115.81
Mean tertiary follicle diameter (MGFD, µm)	221.56 ± 5.97a	269.82 ± 9.35b	305.38 ± 4.12c	438.67 ± 10.33d	140.54
Histomorphometric analysis of the morphological changes of seminiferous tubules and enumeration of germ cells and Leydig cells – F1 generation male ††					
Johnsen's mean testicular biopsy score (JTBS)	9.34 ± 0.42a	7.56 ± 0.56b	4.98 ± 0.15c	3.04 ± 0.11d	58.914
Mean diameter of seminiferous tubule (MSTD, µm)	223.85 ± 1.05a	206.52 ± 1.59b	178.89 ± 1.88c	146.93 ± 5.47d	122.01
Seminiferous tubule epithelial height (STEH, µm)	87.92 ± 2.11a	75.11 ± 3.06b	68.35 ± 1.08b	44.87 ± 1.93c	69.704
Interstitial space (IS, µm)	3.31 ± 0.11a	5.08 ± 0.16b	6.83 ± 0.46c	9.01 ± 0.43d	54.716
Tunica albuginea thickness (TAT, µm)	21.86 ± 0.76a	18.15 ± 0.45b	13.72 ± 0.33c	8.51 ± 0.15d	145.53

Tubular lumen (TL, µm)	4.41 ± 0.14a	5.23 ± 0.17b	6.11 ± 0.23c	7.32 ± 0.11d	54.735
Testis tissue damage score	0a	1.99 ± 0.21b	2.36 ± 0.05b	5.98 ± 0.65c	52.904
No. of Spermatogonia cells	55.76 ± 1.55a	46.13 ± 1.98b	22.76 ± 0.58c	19.37 ± 1.94c	120.88
No. of spermatocytes	83.17 ± 1.53a	61.07 ± 1.88b	42.91 ± 2.32c	21.44 ± 2.05d	178.55
No. of spermatids	76.90 ± 1.34a	58.27 ± 1.05b	38.04 ± 2.45c	22.56. ± 0.67d	240.09
No. of Sertoli cells	12.15 ± 0.45a	10.33 ± 0.31b	8.21 ± 0.08c	4.38 ± 0.42d	92.629
No. of Leydig cells	9.11 ± 0.11a	6.55 ± 0.14b	5.01 ± 0.44c	2.93 ± 0.17d	106.69

Values are expressed as mean ± standard error mean (n = 5/treatment group). Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different letters column wise indicate that treatment groups are significantly different at $P < 0.0001$ and with similar letters indicate that treatment groups are not statistically significant at $P > 0.05$.

† n = 5/treatment group and $F_{3,19}$; †† n = 10 fields/treatment group and $F_{3,39}$.

OTDS - Normal: score - 0; < 25% damage: score 1; 25 - 50% damage: score 2; 50 - 75% damage: score 3; 75 - 100% damage: score 4.

OBS - Score 10: Complete oogenesis with abundant ovarian follicles; Score 9: Many primordial and primary and with irregular germinal epithelium; Score 8: Less number of secondary follicles and corpus luteum; Score 7: No mature Graafian follicles, but many primordial follicles; Score 6: Few primordial follicles; Score 5: Few Graafian follicles with few atretic follicles; Score 4: Many atretic follicles with few Graafian follicles; Score 3: Absence of corpus luteum; Score 2: Presence of many atretic follicles; Score 1: Vacuolated ovarian cortex and medulla with complete oogenesis arrest.

Fig. S1

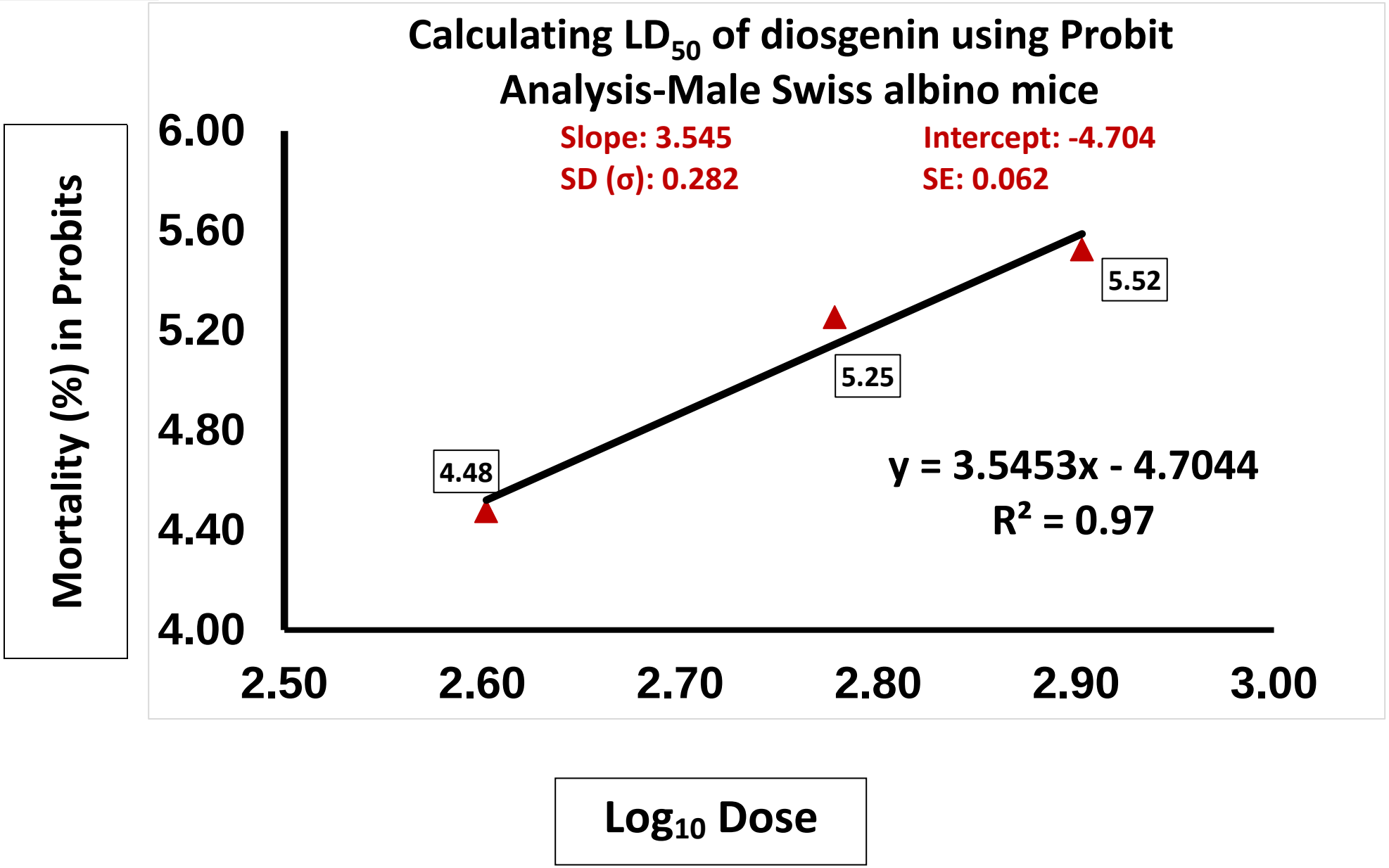


Fig. S1 Estimation of lethal dose (LD_{50}) of diosgenin in male Swiss albino mice using probit method.

Fig. S2

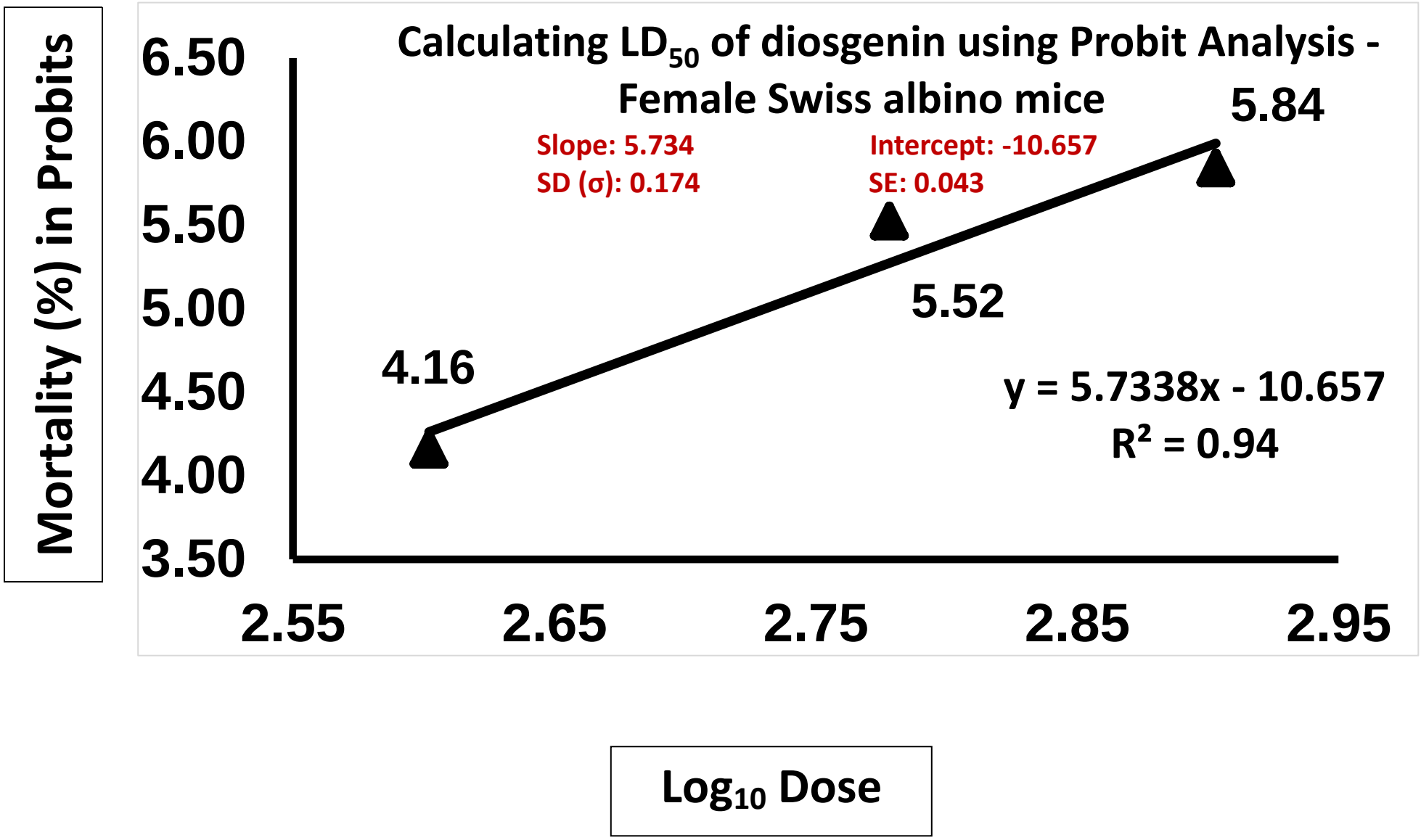


Fig. S2 Estimation of lethal dose (LD_{50}) of diosgenin in female Swiss albino mice using probit method.

Fig. S3

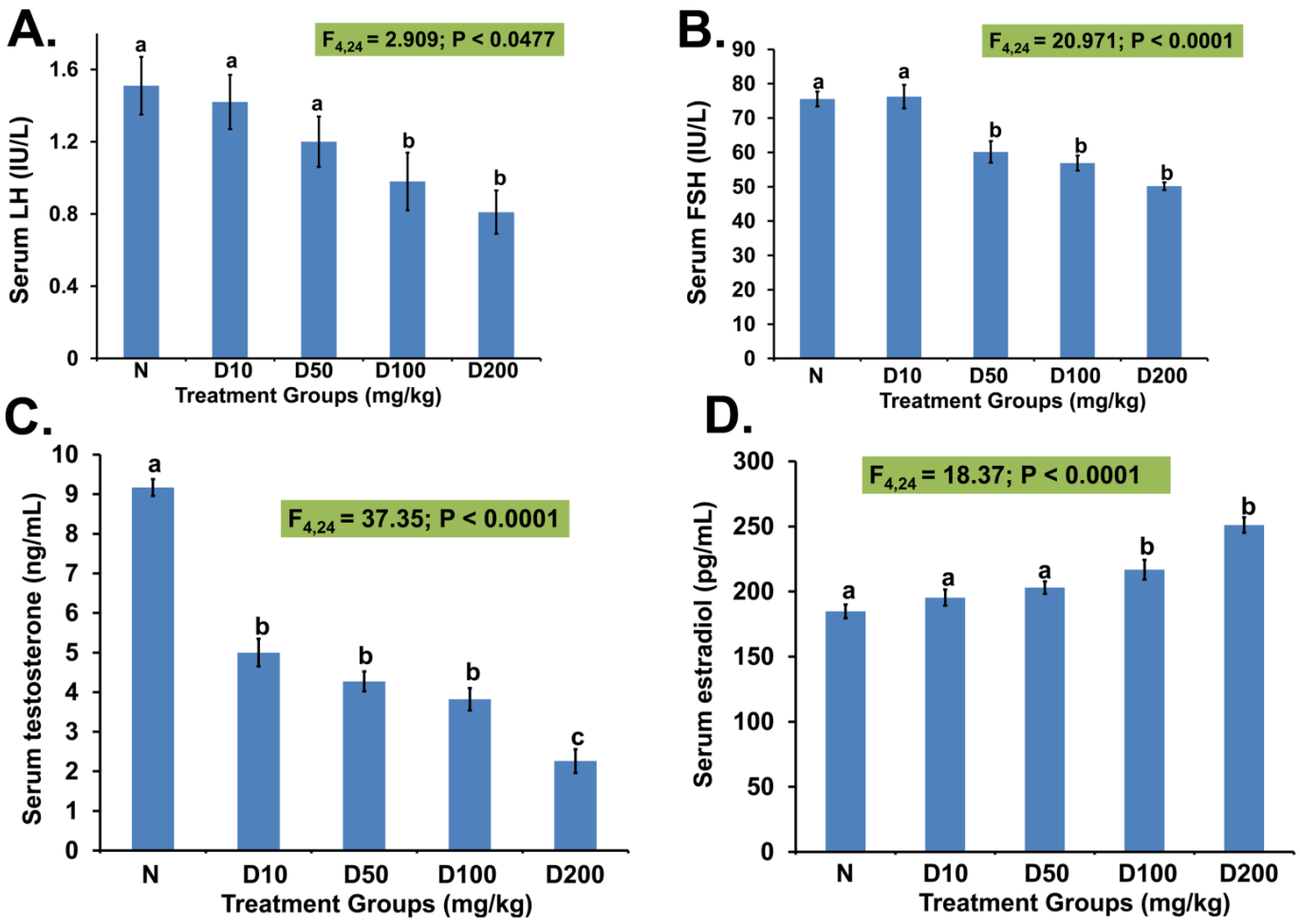


Fig. S3 Long-term feeding effects of diosgenin on the serum reproductive hormone levels in the male Swiss albino mice after repeated dose 90-day oral toxicity study. (A) Serum luteinizing hormone levels (LH, IU/L). (B) Serum follicle stimulating hormone levels (FSH, IU/L). (C) Serum testosterone levels (ng/mL). (D) Serum estradiol levels (pg/mL). Values are expressed as mean ± standard error mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar diagram with different letter means statistical significant difference at $p < 0.05$ and similar letters are not significant. N, Normal untreated mice administered orally with isotonic saline (0.9%) containing Tween 80 for 40 days using intragastric tube; D10, D50, D100, D200, Diosgenin treatment group mice received 10, 50, 100 and 200 mg/kg/day diosgenin dissolved in 0.9% saline solution containing Tween 80 administered orally for 40 days using intragastric tube, respectively.

Fig. S4

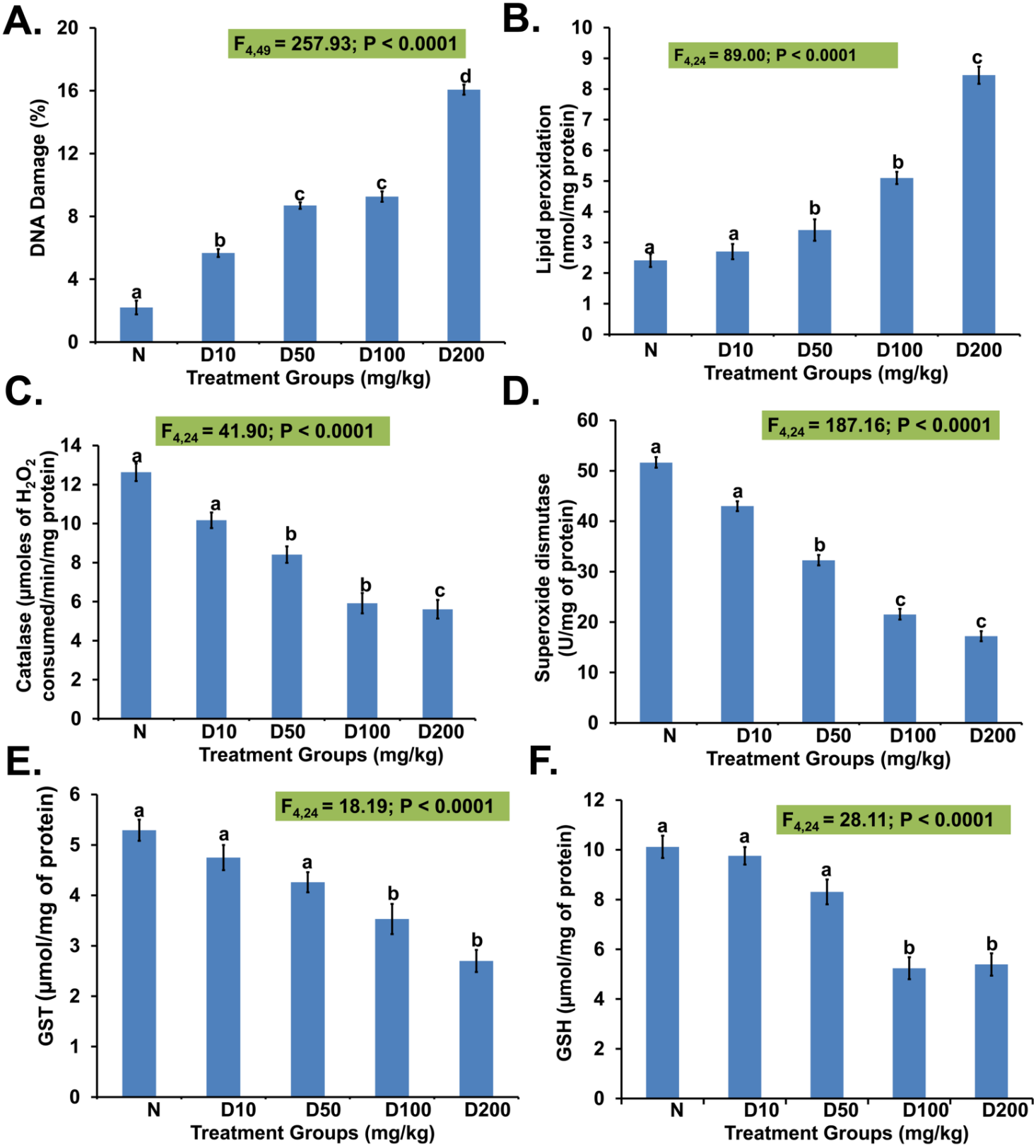

A.
$F_{4,49}$ = 257.93; P < 0.0001
DNA Damage (%)
Treatment Groups (mg/kg)
N
D10
D50
D100
D200
B.
$F_{4,24}$ = 89.00; P < 0.0001
Lipid peroxidation (nmol/mg protein)
C.
$F_{4,24}$ = 41.90; P < 0.0001
Catalase (µmoles of H_2O_2 consumed/min/mg protein)
D.
$F_{4,24}$ = 187.16; P < 0.0001
Superoxide dismutase (U/mg of protein)
E.
$F_{4,24}$ = 18.19; P < 0.0001
GST (µmol/mg of protein)
F.
$F_{4,24}$ = 28.11; P < 0.0001
GSH (µmol/mg of protein)

Fig. S4 Long-term feeding effects of diosgenin on the sperm DNA damage, oxidative stress in testis and depletion of antioxidant enzymes reserve in the male Swiss albino mice after repeated dose 90-day oral toxicity study. (A) Sperm DNA damage (%). (B) Lipid peroxidation (MDA levels, nmol/mg of protein) in testis tissue. (C) Catalase activity (CAT, H_2O_2 denegation at 240 nm, µmoles of H_2O_2 consumed/min/mg protein) in testis tissue. (D) Superoxide dismutase activity in testis tissue (SOD, quantified 50% inhibition of nitro blue tetrazolium with superoxide dismutase at 580 nm, U/mg). (E) Glutathione S-transferase (GST, interaction of 1-chloro-2,4-dinitrobenzene with S-2,4-dinitro-phenyl glutathione at 340 nm, µmoles/mg of protein). (F) Reduced glutathione (GSH, interaction of 5-5-dithio-bis(2-nitrobenzoic acid) at 412 nm, µmoles/mg of protein). Values are expressed as mean ± standard error mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistical significant difference at $p < 0.05$ and similar letters are not significant. N, Normal untreated mice administered orally with isotonic saline (0.9%) containing Tween 80 for 40 days using intragastric tube; D10, D50, D100, D200, Diosgenin treatment group mice received 10, 50, 100 and 200 mg/kg/day diosgenin dissolved in 0.9% saline solution containing Tween 80 administered orally for 40 days using intragastric tube, respectively.

Fig. S5

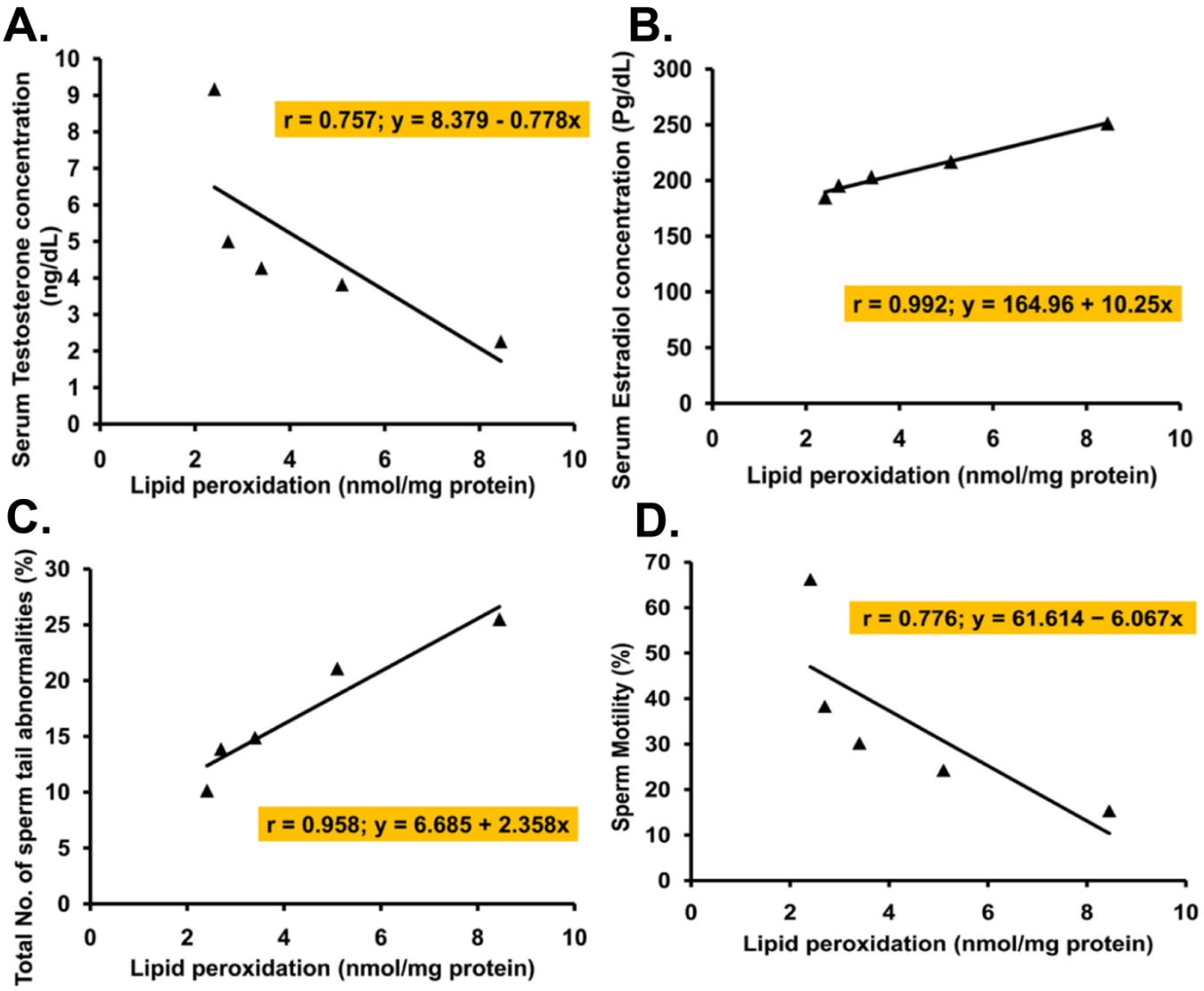


Fig. S5 Correlation and regression analysis between oxidative stress, hormonal imbalance and sperm quality (repeated dose 90-day oral toxicity study). (A) Lipid peroxidation product (MDA level, nmol/mg of protein) vs. serum testosterone level [ng/dL]. (B) Lipid peroxidation product (MDA level, nmol/mg of protein) vs. serum estradiol [pg/dL] level. (C) Lipid peroxidation product (MDA level, nmol/mg of protein) vs. sperm tail abnormities [%].(D) Lipid peroxidation product (MDA level, nmol/mg of protein) vs. sperm motility [%].

Fig. S6

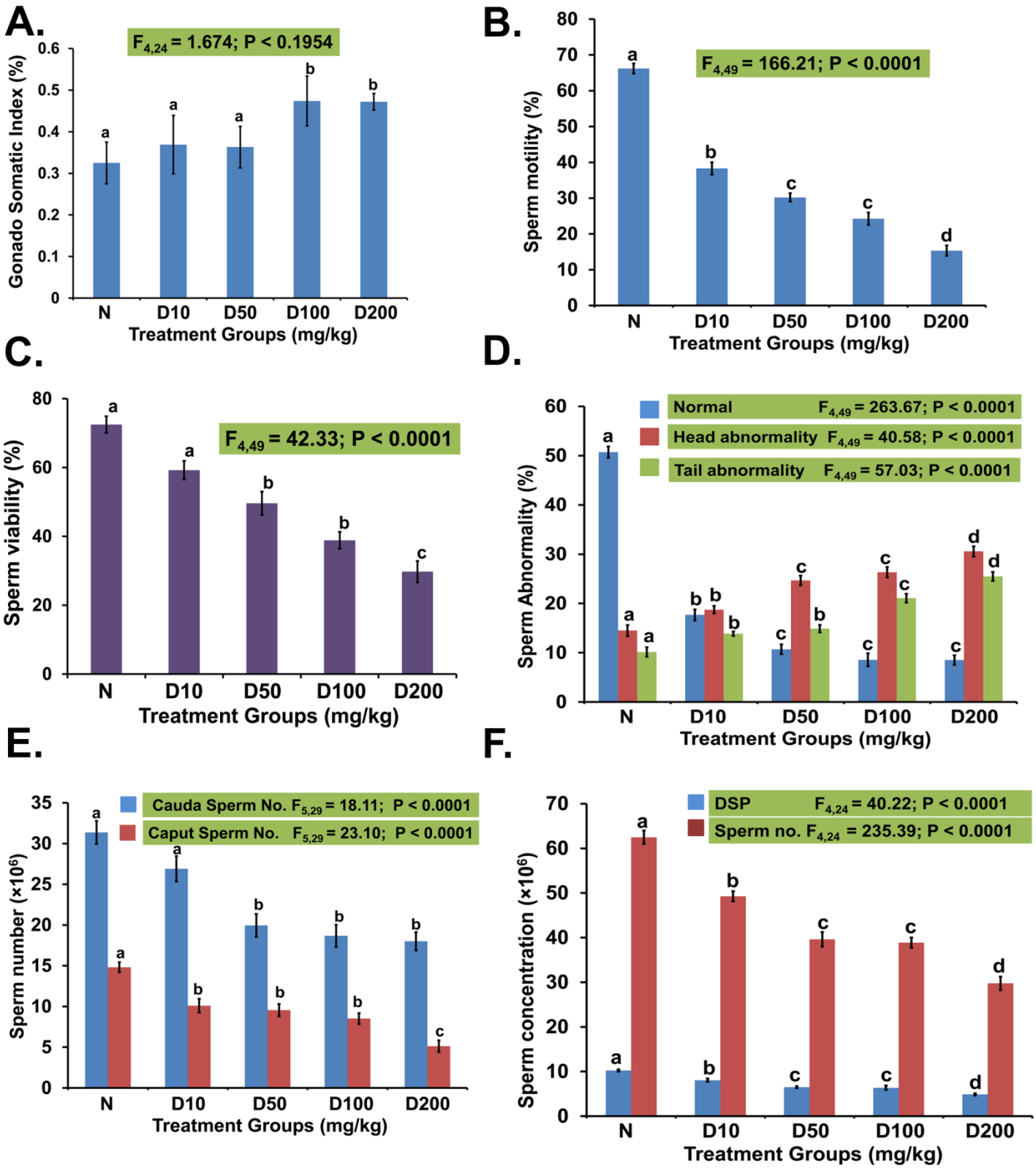
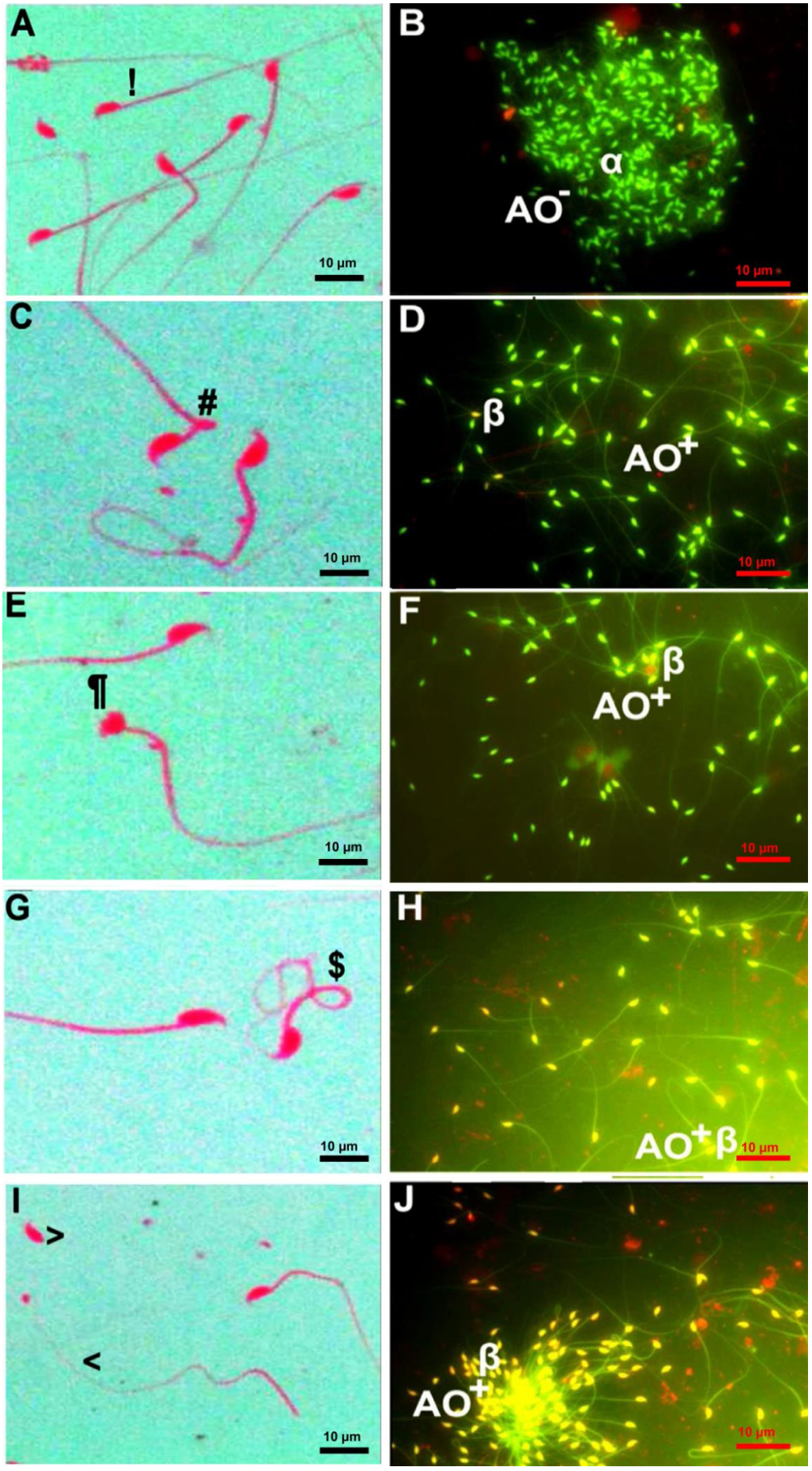

A.
$F_{4,24}$ = 1.674; P < 0.1954
Gonado Somatic Index (%)
Treatment Groups (mg/kg)
N
D10
D50
D100
D200
B.
$F_{4,49}$ = 166.21; P < 0.0001
Sperm motility (%)
C.
$F_{4,49}$ = 42.33; P < 0.0001
Sperm viability (%)
D.
Normal $F_{4,49}$ = 263.67; P < 0.0001
Head abnormality $F_{4,49}$ = 40.58; P < 0.0001
Tail abnormality $F_{4,49}$ = 57.03; P < 0.0001
Sperm Abnormality (%)
E.
Cauda Sperm No. $F_{5,29}$ = 18.11; P < 0.0001
Caput Sperm No. $F_{5,29}$ = 23.10; P < 0.0001
Sperm number (×10⁶)
F.
DSP $F_{4,24}$ = 40.22; P < 0.0001
Sperm no. $F_{4,24}$ = 235.39; P < 0.0001
Sperm concentration (×10⁶)

Fig. S6 Long-term feeding effects of diosgenin on the sperm functional indices in the male Swiss albino mice after repeated dose 90-day oral toxicity study. (A) Gonado somatic index (%). (B) Sperm motility [%]. (C) Sperm viability [%]. (D) Sperm head and tail abnormalities [%]. (E) Number of spermatozoa in cauda and caput epididymis ($\times 10^6$). (F) Sperm concentration ($\times 10^6$) in testis in terms of daily sperm production [DSP] and sperm number (F). Values are expressed as mean ± standard error mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistical significant difference at $p < 0.05$ and similar letters are not significant. GSI, [(testicular weight/body weight) × 100]; N, Normal untreated mice administered orally with isotonic saline (0.9%) containing Tween 80 for 40 days using intragastric tube; D10, D50, D100, D200, Diosgenin treatment group mice received 10, 50, 100 and 200 mg/kg/day diosgenin dissolved in 0.9% saline solution containing Tween 80 administered orally for 40 days using intragastric tube, respectively.

Fig. S7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AO+
10 µm

Fig. S7 Long-term feeding effects of diosgenin on the defects in sperm morphology (eosin staining) and sperm DNA damage (acridine orange test) in the male Swiss albino mice after repeated dose 90-day oral toxicity study. × 40 eye piece magnification. (A, C, E, G, I) Observations of normal sperms in the untreated control mice group and sperm head and tail abnormalities in diosgenin treated mice groups at a dose of 10, 50, 100, 200 mg/kg, respectively. (B, D, F, H, J) Observations of normal native double-stranded DNA (green fluorescent, AO^-) in untreated control mice group and denatured single-stranded DNA (orange/red fluorescent, AO^+) in diosgenin treated mice groups at a dose of 10, 50, 100, 200 mg/kg, respectively. Scale bar = 10 µm. **!,** Normal sperm head and tail; **#**, Banana and folded head; **¶**, Amorphous and hookless head; **$,** Coiled tail; >, Detached head; <, Broken tail; **α,** Normal sperm DNA; **β,** Damaged sperm DNA.

Fig. S8

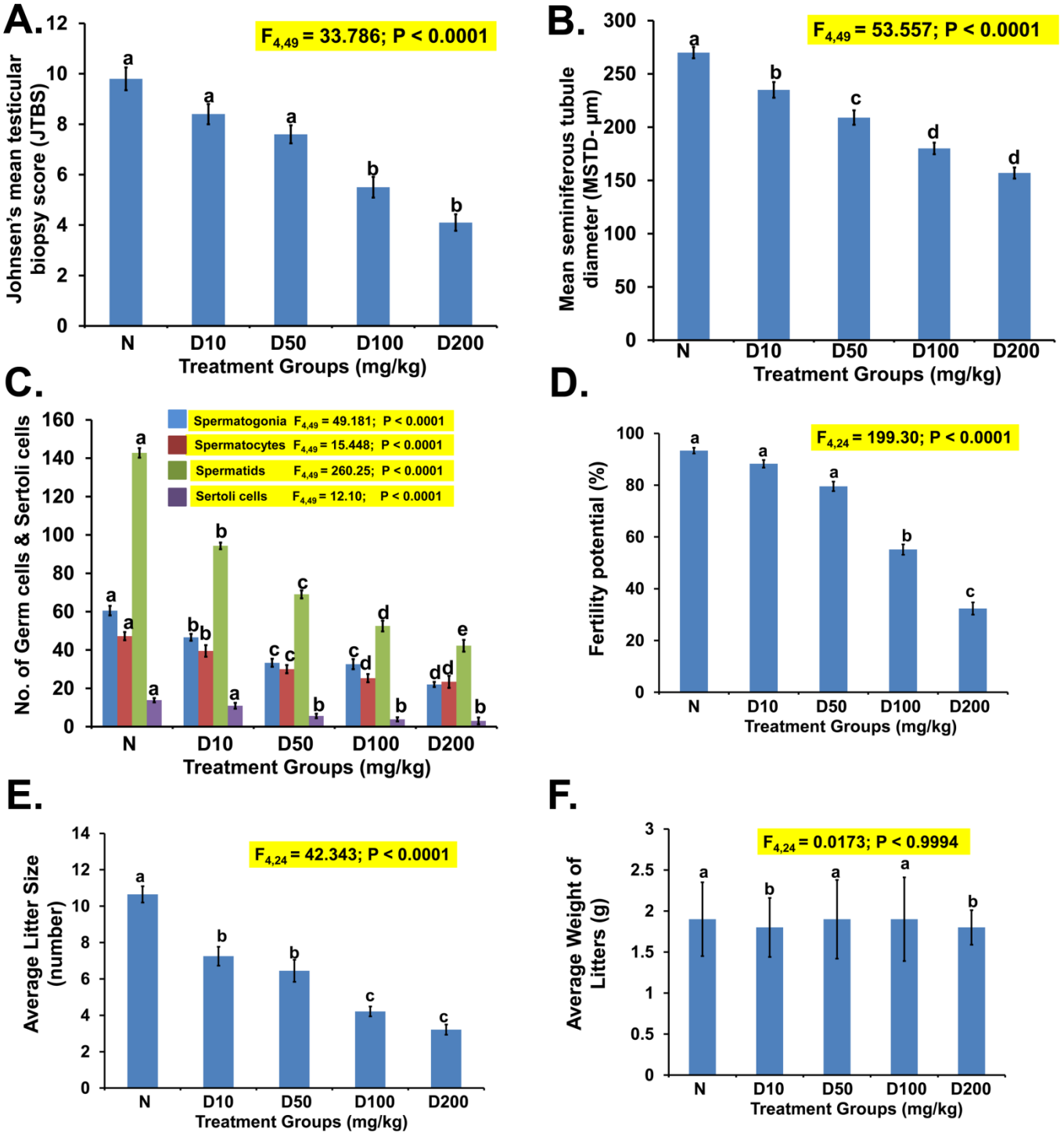

A.
$F_{4,49}$ = 33.786; P < 0.0001
Johnsen's mean testicular biopsy score (JTBS)
Treatment Groups (mg/kg)
N
D10
D50
D100
D200
B.
$F_{4,49}$ = 53.557; P < 0.0001
Mean seminiferous tubule diameter (MSTD- µm)
Treatment Groups (mg/kg)
C.
Spermatogonia $F_{4,49}$ = 49.181; P < 0.0001
Spermatocytes $F_{4,49}$ = 15.448; P < 0.0001
Spermatids $F_{4,49}$ = 260.25; P < 0.0001
Sertoli cells $F_{4,49}$ = 12.10; P < 0.0001
No. of Germ cells & Sertoli cells
Treatment Groups (mg/kg)
D.
$F_{4,24}$ = 199.30; P < 0.0001
Fertility potential (%)
Treatment Groups (mg/kg)
E.
$F_{4,24}$ = 42.343; P < 0.0001
Average Litter Size (number)
Treatment Groups (mg/kg)
F.
$F_{4,24}$ = 0.0173; P < 0.9994
Average Weight of Litters (g)
Treatment Groups (mg/kg)

Fig. S8 Repeated dose effects of diosgenin (90-day oral toxicity study) on spermatogenesis, testis function and fertility in the Swiss albino mice. (A) Johnson's mean testicular biopsy score [JTBS]. (B) Histomorphometric changes on mean seminiferous tubules diameter [MSTD, μm]. (C) Quantification of number of germ cells and Sertoli cells in seminiferous tubules. (D) Fertility potential [%]. (E) Mean number of litter size. (F) Mean weight of the litters [g]. Values are expressed as mean ± standard error mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistical significant difference at $P < 0.05$ and similar letters are not significant. N, Normal untreated mice administered orally with isotonic saline (0.9%) containing Tween 80 for 90 days using intragastric tube; D10, D50, D100, D200, Diosgenin treatment group mice received 10, 50, 100 and 200 mg/kg/day dissolved in 0.9% saline solution containing Tween 80 administered orally for 90 days using intragastric tube, respectively.

Fig. S9

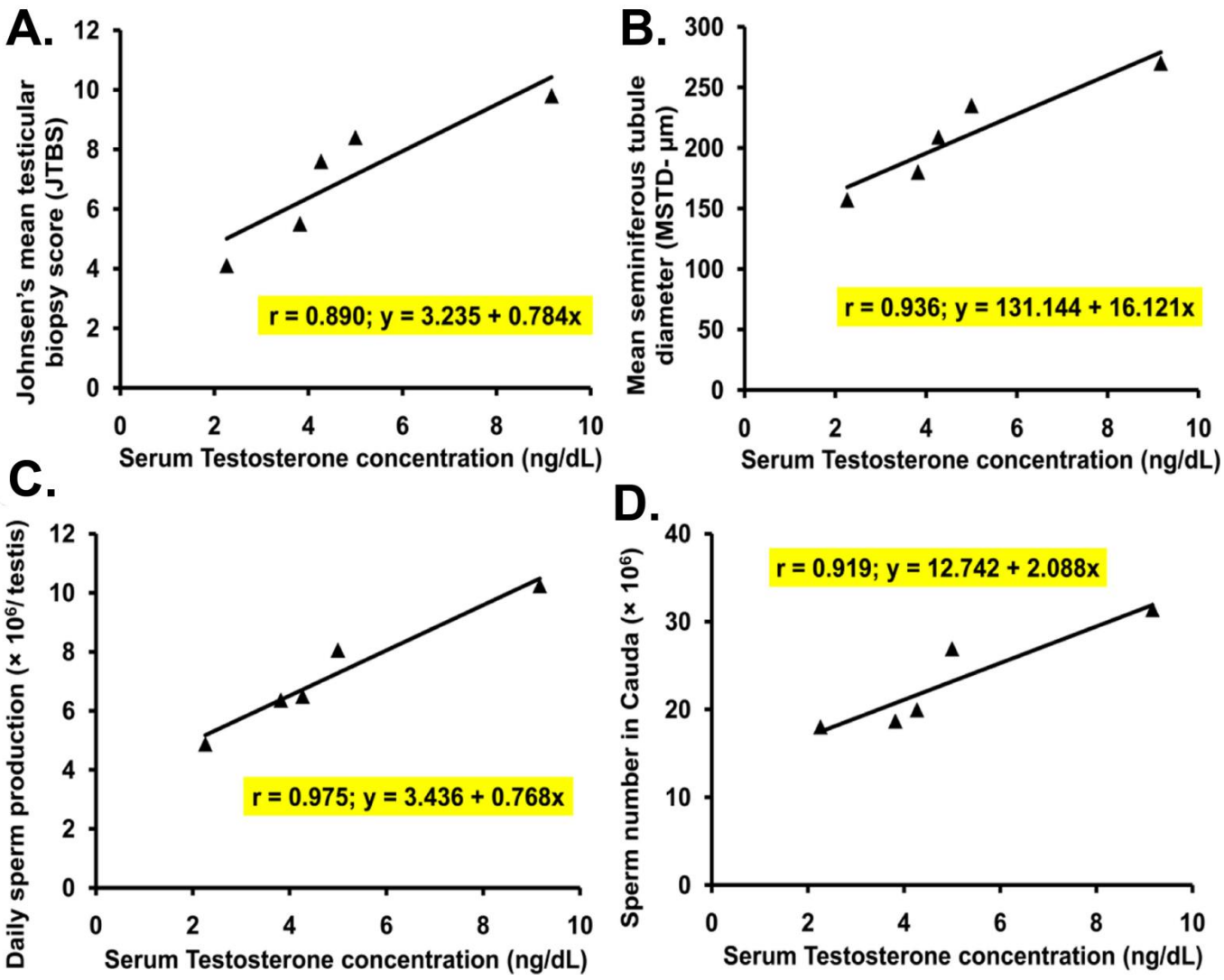


Fig. S9 Correlation and regression analysis between serum testosterone concentration [ng/dL], testis damage, histo-morphometric changes in testis and sperm quality parameters (repeated dose 90-day oral toxicity study). (A) Serum testosterone vs. Johnson's mean testicular biopsy score [JTBS]. (B) Serum testosterone vs. mean seminiferous tubule diameter [MSTD, µm]. (C) Serum testosterone vs. daily sperm production [DSP, × 10^6/testis]. (D) Serum testosterone vs. cauda epididymis sperm concentration [× 10^6/cauda epididymis].

Fig. S10

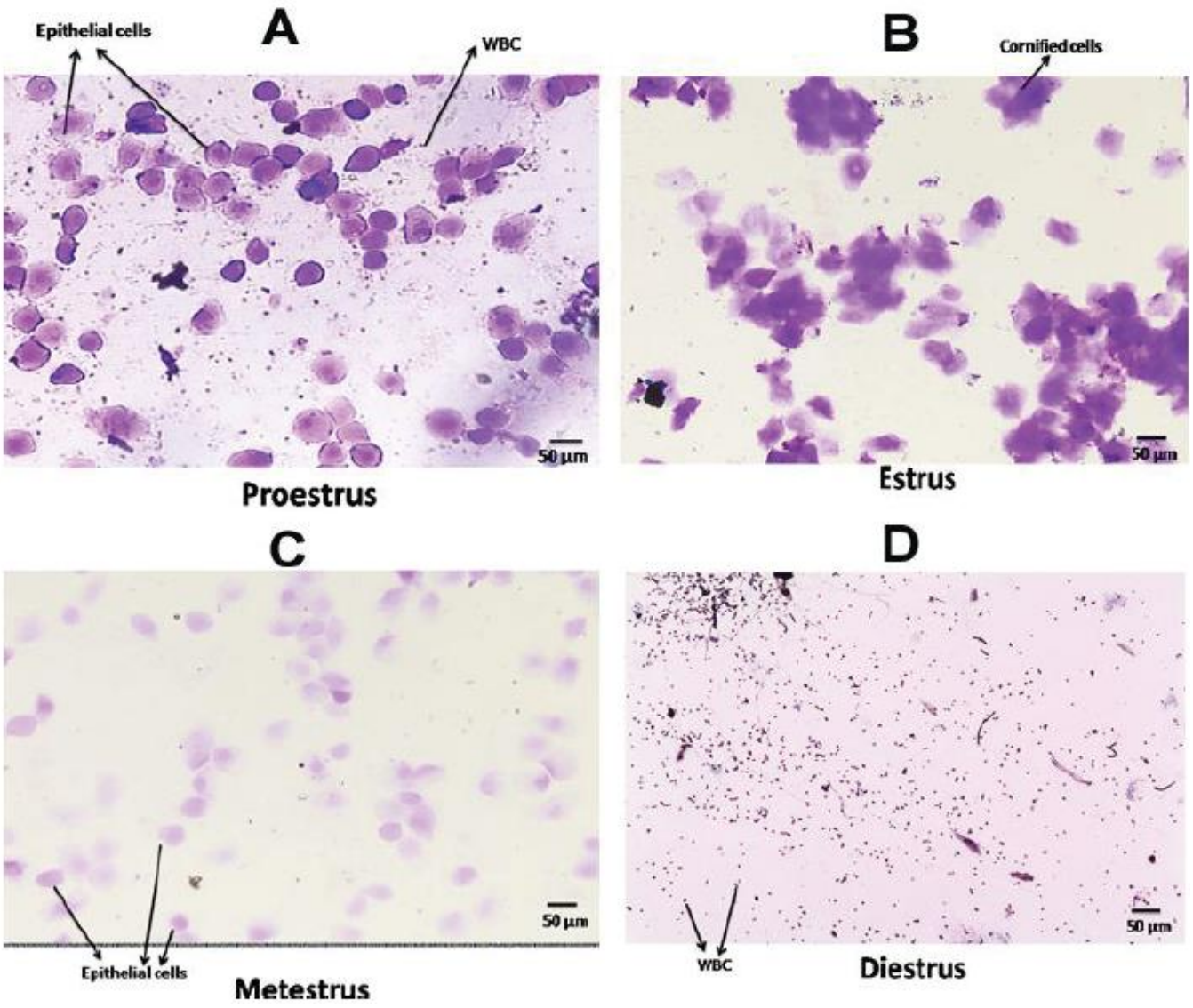
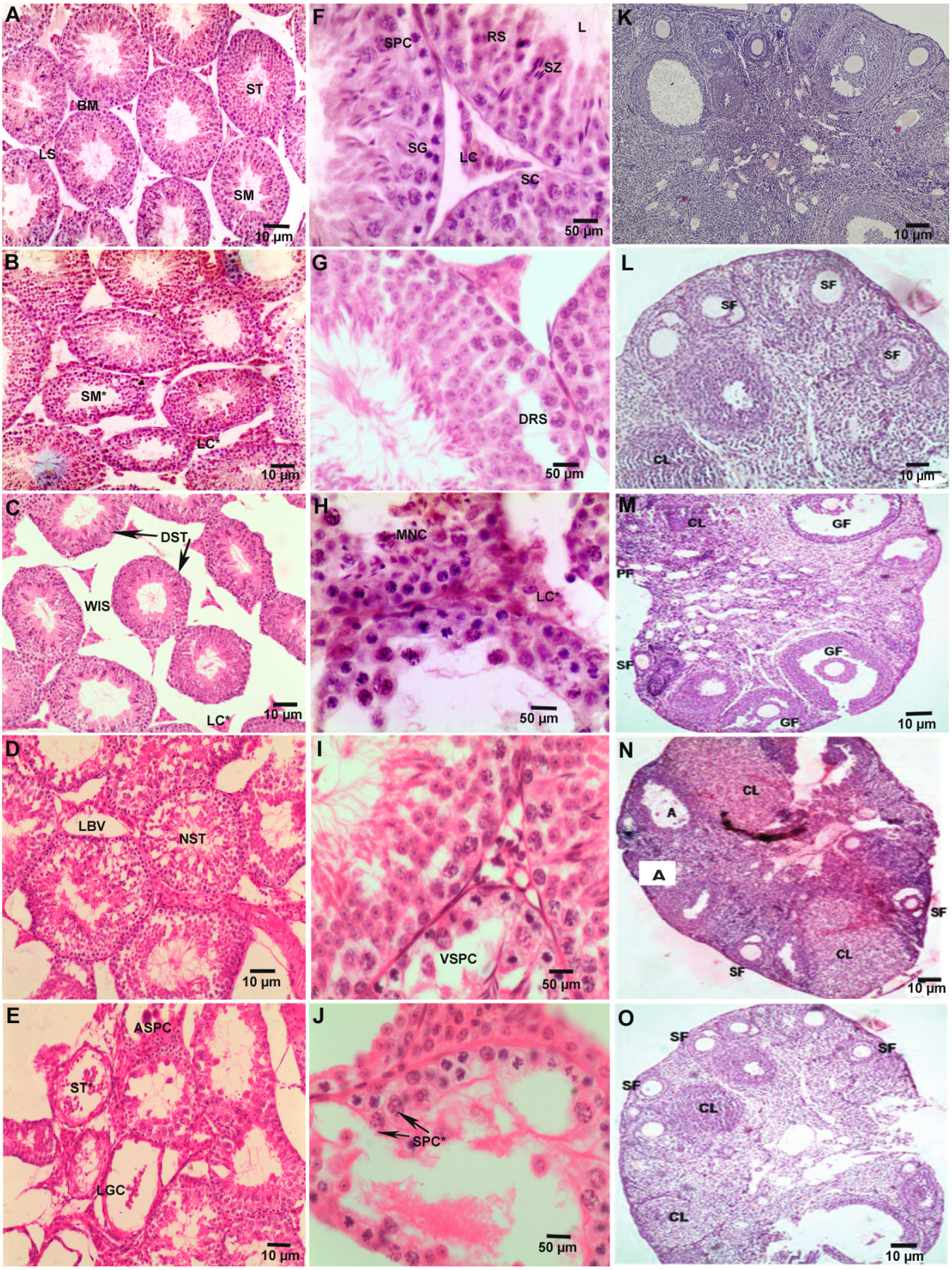


Fig. S10 Vaginal smears in each stage of mice estrus cycle. (A) Proestrous stage displaying mainly nucleated epithelial cells and parvus keratinized cells, b) c) and d) diestrus stage showing almost exclusively leukocytes. (B) Estrous stage exhibiting large anucleated cornified cells. (C) Metestrous stage presenting approximately equal numbers of leukocytes, cornified cells, and nucleated epithelial cells. (D) Diestrus stage showing almost exclusively leukocytes.

Fig. S11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LC*
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DST
WIS
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D
LBV
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LGC
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RS
L
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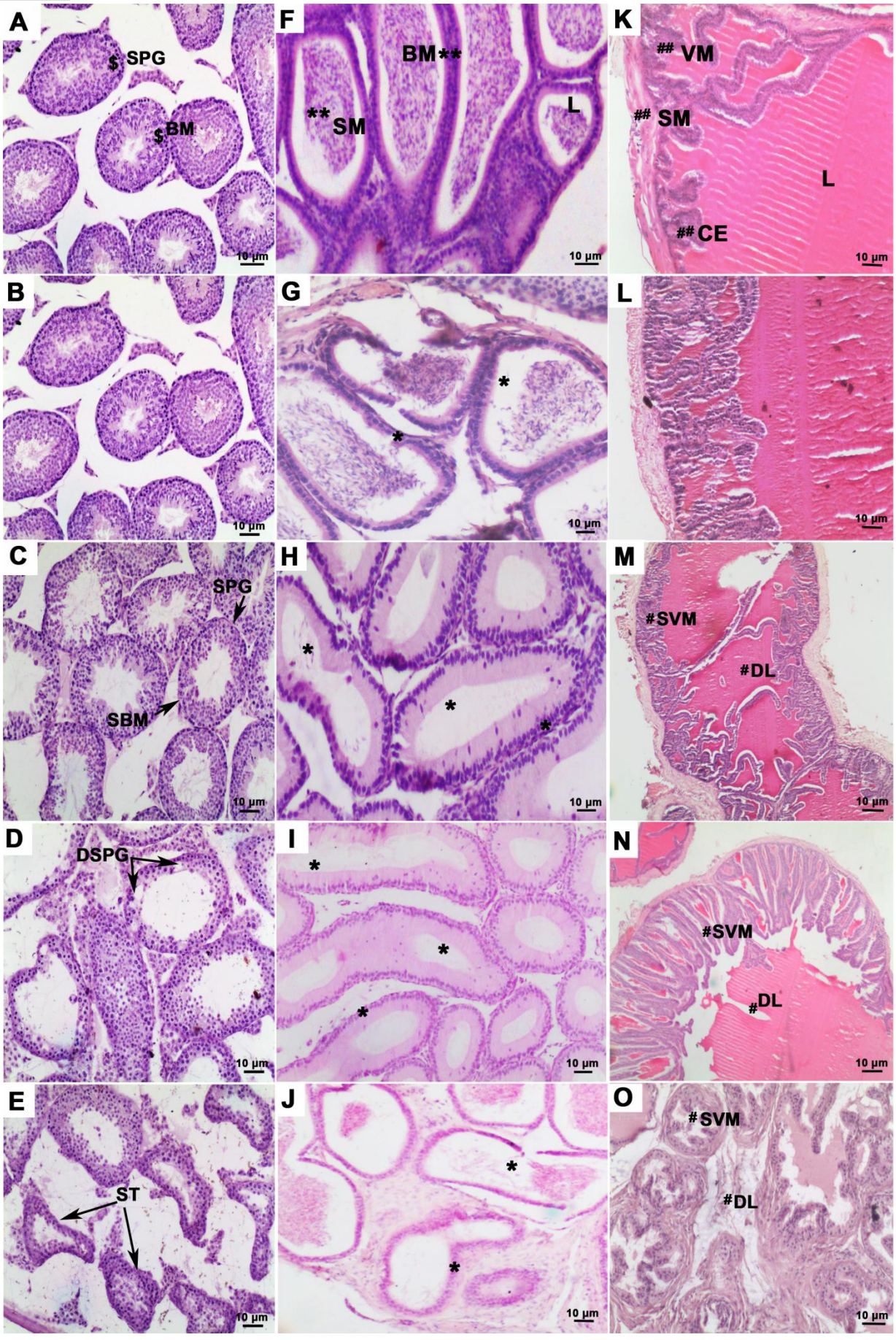
Fig. S11 Long-term feeding effects of diosgenin on testis and ovary histopathological anomalies in the male and female Swiss albino mice after repeated dose 90-day oral toxicity study. Hematoxylin and eosin staining (H&E), × 10 (A-E, K-O) and × 40 (F-J) eyepiece magnifications, scale bar = 10 and 50 μm, respectively. Untreated control group testis tissue sections (A, F), showing intact normal testis histological characteristics with an ideal sequence of arranged cells in spermatogenic sequence while diosgenin treated group testis tissue sections at a dose of 10 mg/kg (B, G), 50 mg/kg (C, H), 100 mg/kg (D, I), and 200 mg/kg (E, J), demonstrating atrophy, necrotic cells, sloughing of germ cells, vacuolization, wider inter intestinal area between seminiferous tubules and germ cell depletion and degeneration. Histological section of the control mice ovary (K) showing the presence of normal Graafian follicle (GF), plenty of primary (PF), secondary follicles (SF), and corpora lutea (CL) whereas, diosgenin treated mice ovary (L-O) displaying few GF, PF, SF, and CL and many atretic follicles (A) and pyknotic cells (arrow). The magnitude of the histopathological alterations was increased with the concentrations of diosgenin. ASPC, Apoptotic spermatocytes; BM, Basement membrane; ST, seminiferous tubules; ST*, No spermatogenesis in seminiferous tubules; SG: spermatogtonia, SPC: Presence of spermatocytes, SPC*: Mitotic arrest in spermatocytes, IS: interstitial space, IBM: intact basement membrane, L: Lumen, SM: Presence of sperm mass, SM*: Few or no sperm mass, LC: Presence of Leydig cells, LC*: Few or no Leydig cells, SC: Sertoli cells, SZ: Spermatozoa, ES: elongated spermatids, RS: round spermatids, DRS: distorted spermatids, MNC: multinucleated germ cells, WIS: wider interstitial space, DST: Distorted and degenerated seminiferous tubules, VSPC: vacuolated spermatocytes, NST: Necrotic seminiferous tubules, LBV: Loss of blood vessels in interstitial space, LGC: Loss of germ cells.

Fig. S12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$ SPG
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N
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#SVM
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Fig. S12 Effects of diosgenin on histological alterations of testicular glycogen content (PAS), cauda epididymis and seminal vesicle in the male Swiss albino mice after repeated dose 90-day oral toxicity study. Periodic Acid-Schiff hematoxylin staining (PAS) of testis, × 10 (A-E), and hematoxylin and eosin staining (H&E) of cauda epididymis (F-J) and seminal vesicle (K-O), × 10 eyepiece magnifications, scale bar = 10 μm, respectively. Untreated control group testis tissue section (A) showing increase in intense of PAS staining in spermatogonia cells and basement membrane of seminiferous tubules while diosgenin treated group testis tissue sections at a dose of 10 mg/kg (B), 50 mg/kg (C), 100 mg/kg (D), and 200 mg/kg (E) indicating decrease in intensity of PAS-positive spermatogonia cells as well as shrinkage of basement membrane in seminiferous tubules. The degree of the PAS staining intensity was in decreasing trend with the increase in the concentrations of diosgenin. H&E staining of cauda epididymis tissue section of the untreated control group (F), showing a thick layer of columnar epithelium and abundance of sperm mass in lumen whereas, diosgenin treated group cauda epididymis tissue sections at a dose of 10 mg/kg (G), 50 mg/kg (H), 100 mg/kg (I), and 200 mg/kg (J), signifying thin and/or damaged/disorganized columnar epithelium, loss or empty of sperm mass in the lumen. Untreated control group seminal vesicle (K) tissue section, specifying thick layer of columnar epithelium with smooth muscles, villous mucosa and many fingers like projection in lumen while diosgenin treated group seminal vesicle tissue sections at a dose of 10 mg/kg (L), 50 mg/kg (M), 100 mg/kg (N), and 200 mg/kg (O), designating decrease in columnar epithelium with shrinkage in villous mucosa and degenerated lumen. BM: Basement membrane, SPG^{+}: PAS-positive spermatogonia cells, SBM^{+}: Shrinkage of the basement membrane, SPG^{-}: Few or no PAS-positive spermatogonia cells, $DSPG^{+}$: Degenerated spermatogonia, ST^{+}: Shrinkage of the tubule with no visible PAS-positive germ cells, CE: Columnar epithelium, SM: Sperm mass, L: Lumen, L^{-}: Empty lumen, +: Few or no sperm mass in the lumen, →: Ruptured columnar epithelium, SM: Smooth muscles, VM: Villous mucosa, SVM: Shrinkage of villous mucosa, DL: Degenerated lumen.

Fig. S13

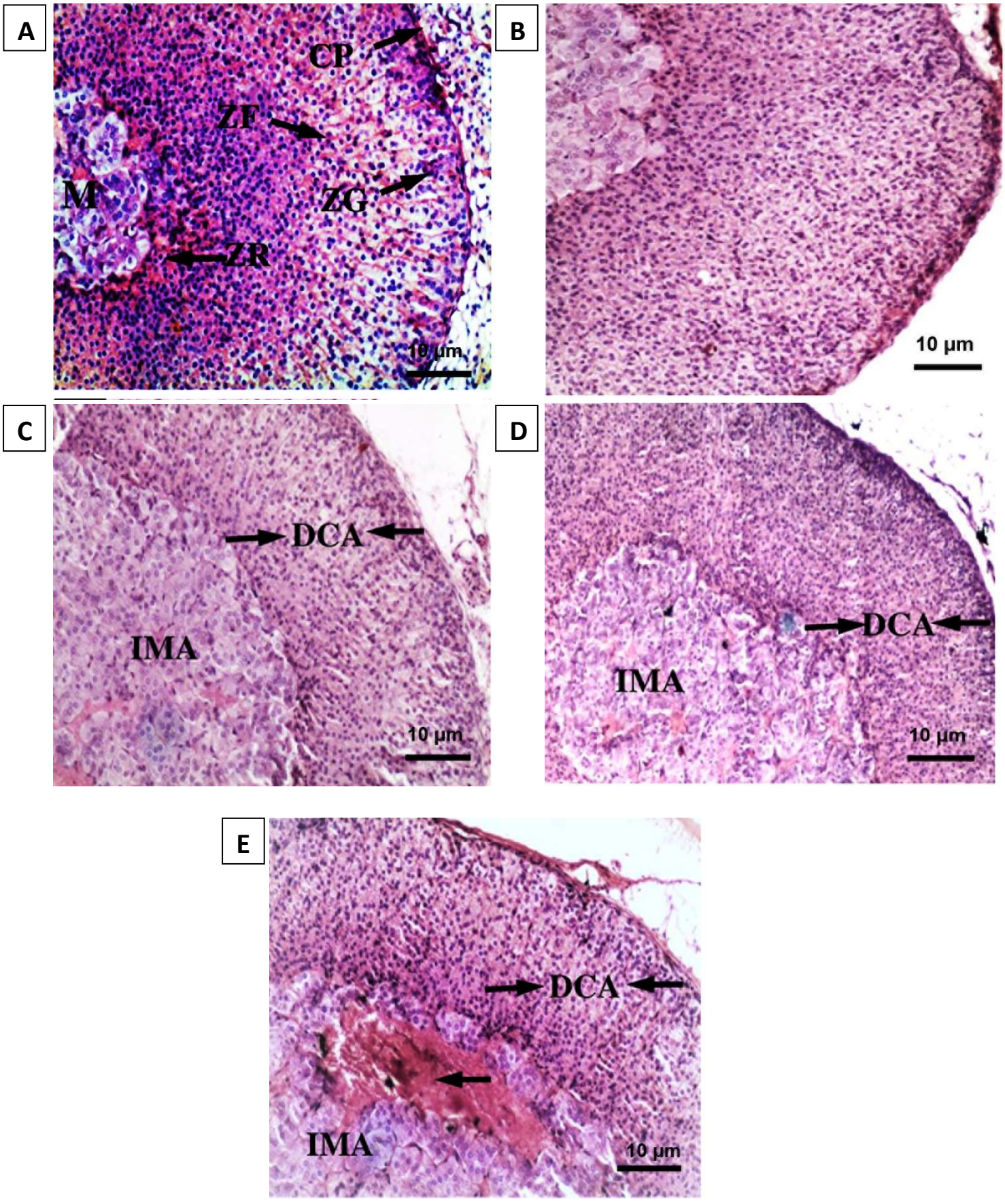
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Fig. S13 Repeated dose effects of diosgenin (90-day oral toxicity study) on the histopathology of adrenal gland of male Swiss albino mice. Hematoxylin and eosin staining (H&E) of adrenal gland × 10 (A-E) eyepiece magnifications, scale bar = 10 μm, respectively. Histological tissue sections of the adrenal gland in untreated control (A) defining balanced tissue contents in adrenocortical region as well in medullar region while diosgenin treated mice at a dose of 10, 50, 100 and 200 mg/kg (B-E) outlining a remarkable decrease in the area of adrenocortical region and a phenomenal increase in the medullar region. CP, Capsule; ZG, Zona glomerulosa; ZF, Zona fasciculate; ZR, Zona reticularis; M, Medulla; DCA, Decreased cortical area; IMA, Increased medulla area.

Fig. S14

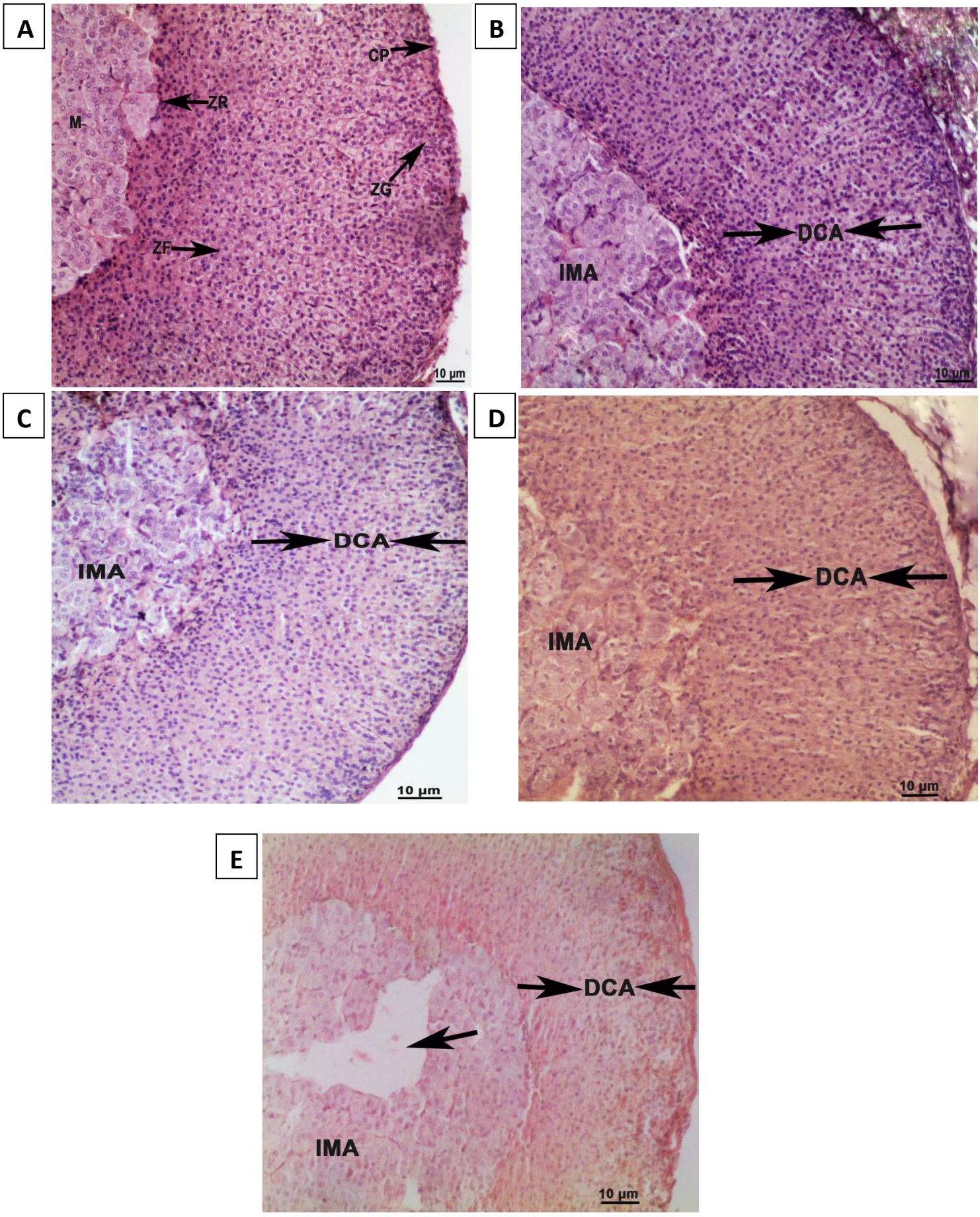
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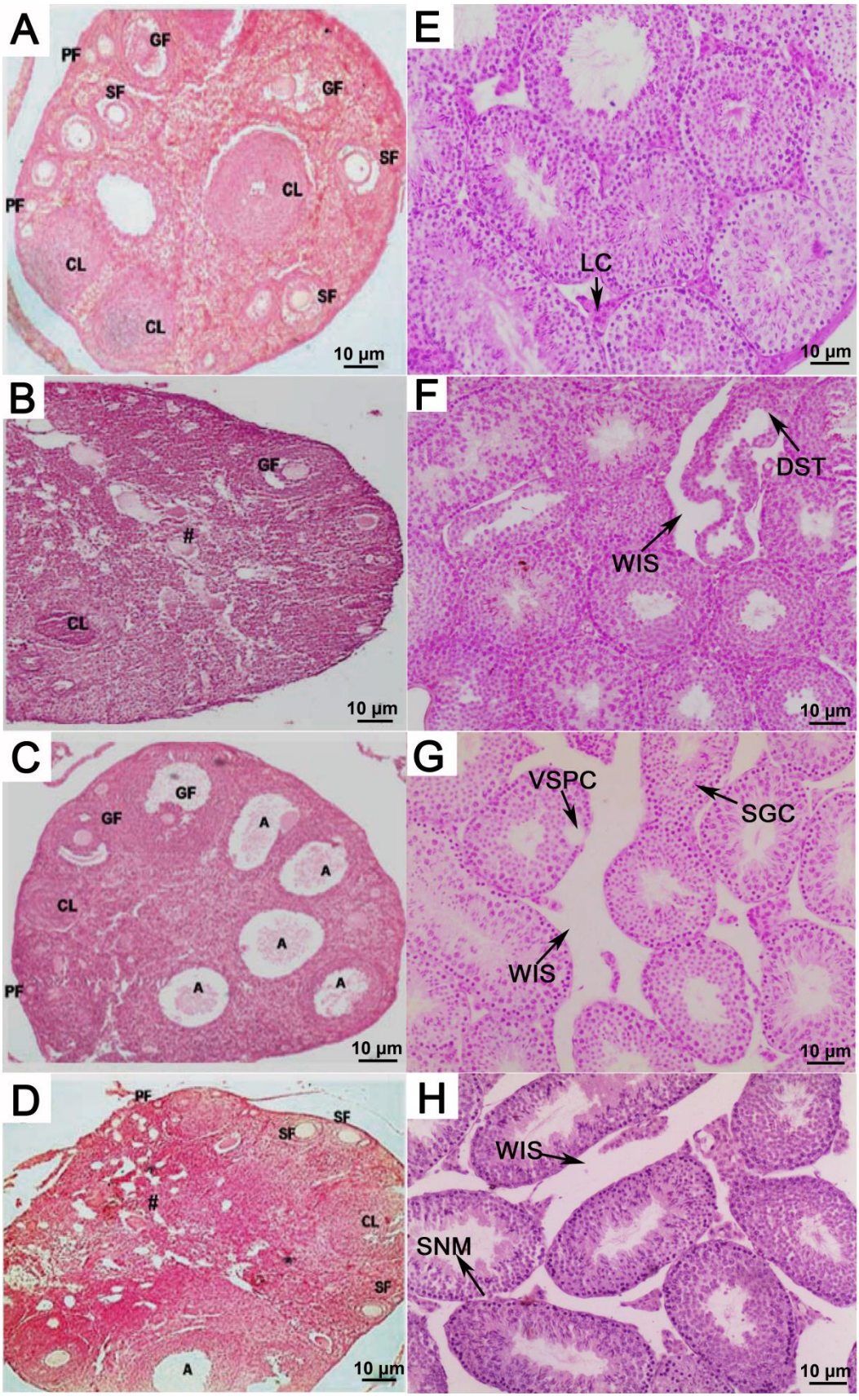
Fig. S14 Repeated dose effects of diosgenin (90-day oral toxicity study) on the histopathology of adrenal gland of female Swiss albino mice. Hematoxylin and eosin staining (H&E) of adrenal gland × 10 (A-E) eyepiece magnifications, scale bar = 10 μm, respectively. Histological tissue sections of the adrenal gland in untreated control (A) defining balanced tissue contents in adrenocortical region as well in medullar region while diosgenin treated mice at a dose of 10, 50, 100 and 200 mg/kg (B-E) outlining a remarkable decrease in the area of adrenocortical region and a phenomenal increase in the medullar region. CP, Capsule; ZG, Zona glomerulosa; ZF, Zona fasciculate; ZR, Zona reticularis; M, Medulla; DCA, Decreased cortical area; IMA, Increased medulla area.

Fig. S15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VSPC
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D
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SF
#
CL
SF
A
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WIS
SNM
10 µm

Fig. S15 Observations of ovarian and testicular histological alterations in the F_1 progeny (F_0 generation exposed to diosgenin) after PND70 (F_1 extended one-generation reproductive toxicity). Hematoxylin and eosin staining (H&E) of ovary (A-D) and testis (E-H), × 10 eyepiece magnifications, scale bar = 10 μm, respectively. (A) Histological section of the ovary of F_1 control mice exhibited normal Graafian follicles (GF), plenty of primordial (PrF), primary (PF), secondary (SF) and tertiary (TF) follicles and corpora lutea (CL). (B-D) Presence of cystic and atretic (A) follicles with pyknotic cells, vacuolization, scanty in PF, GF, and CL were spotted in the F_1 mice ovary (50,100 and 200 mg/kg diosgenin treated F_0 mice), affecting the folliculogenesis. (E-H) Untreated control F_1 mice testis showed normal spermatogenic events while various anomalies were observed [disorientation of seminiferous tubules (DST), wider interstitial spaces (WIS), vacuolated spermatocytes (VSPC), multinucleated spermatogonia (SGC), loss of Sertoli and Leydig cells (LC) and no sperm mass in the lumen (SNM)] in the diosgenin supplemented mice compared with the control.

Fig. S16

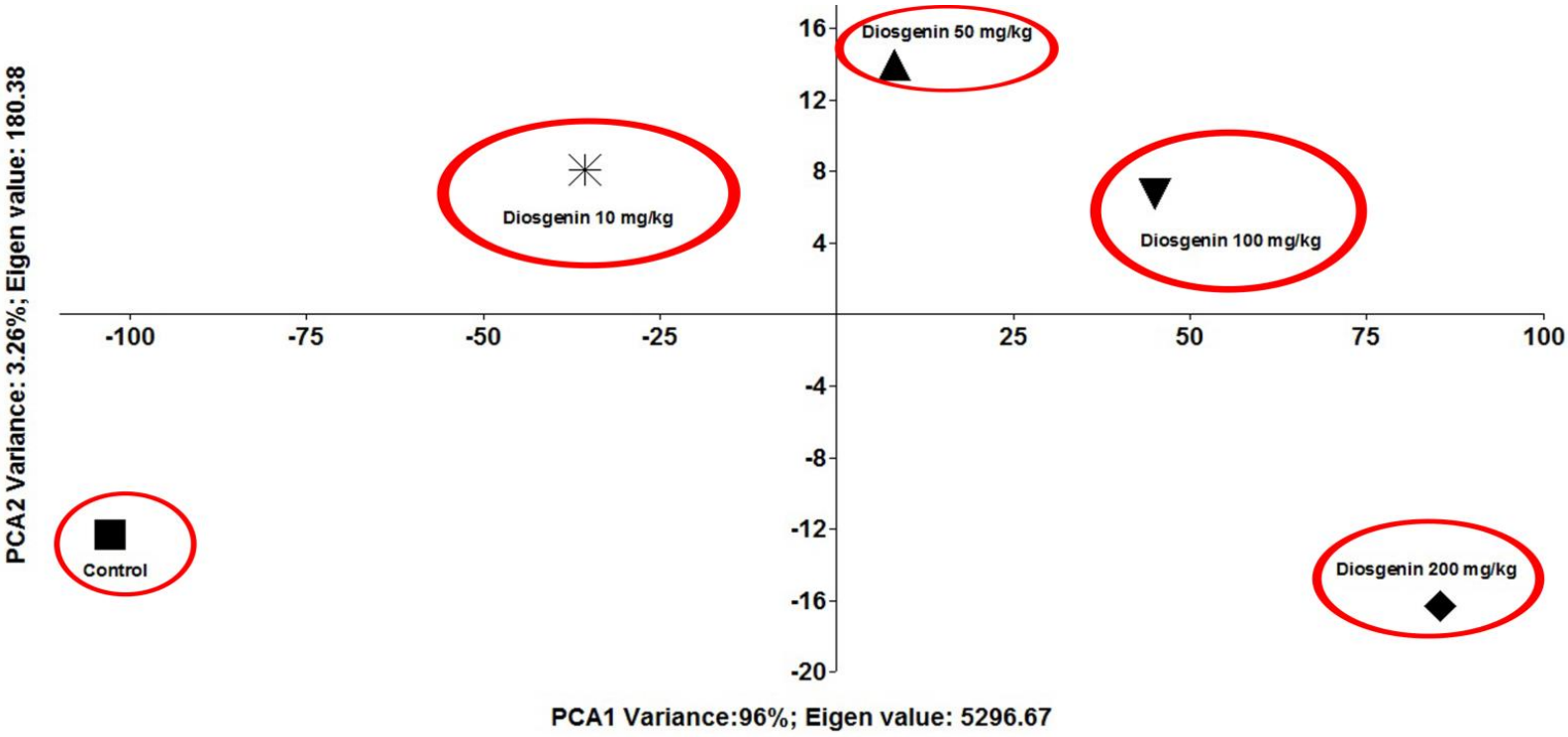

Fig. S16 Principal component analysis (PCA) validates the endocrine disruptor and reproductive toxicant nature of diosgenin upon long-term feeding in male Swiss albino mice (repeated dose 90-day oral toxicity study) by employing the metabolic, physiological and reproductive parameters including reproductive hormones, liver and kidney function profiles, lipid profiles, spermatogenesis, sperm quality, oogenesis, estrous cycle, reproductive performance and markers related to oxidative stress, steroidogenesis, cell proliferation and germ cell apoptosis.

Fig. S17

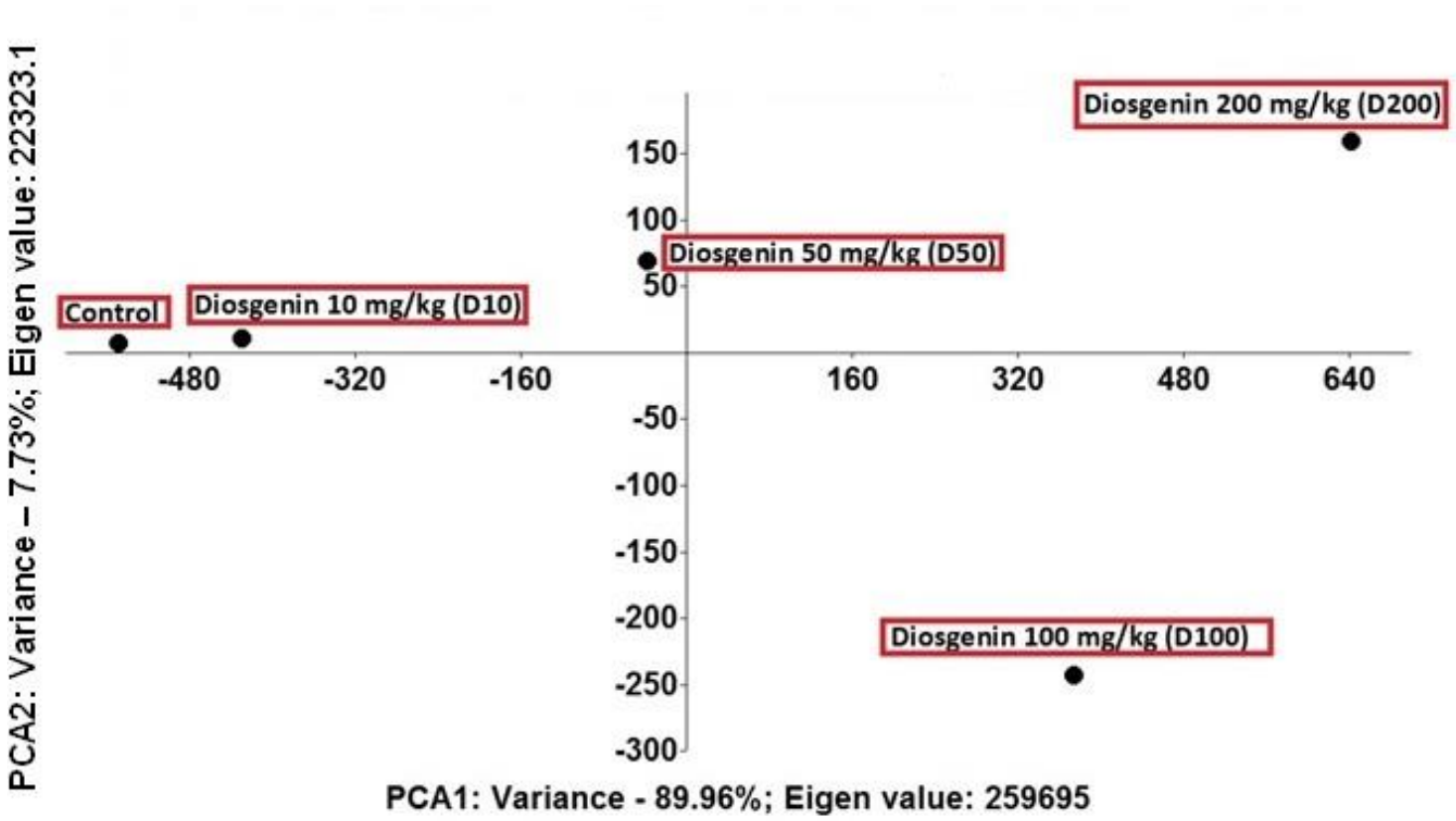

Fig. S17 Principal component analysis (PCA) evaluation of endocrine disruptor and reproductive toxicant potential of diosgenin in female Swiss albino mice (repeated dose 90-day oral toxicity study) using the metabolic, physiological and reproductive parameters.

Fig. S18

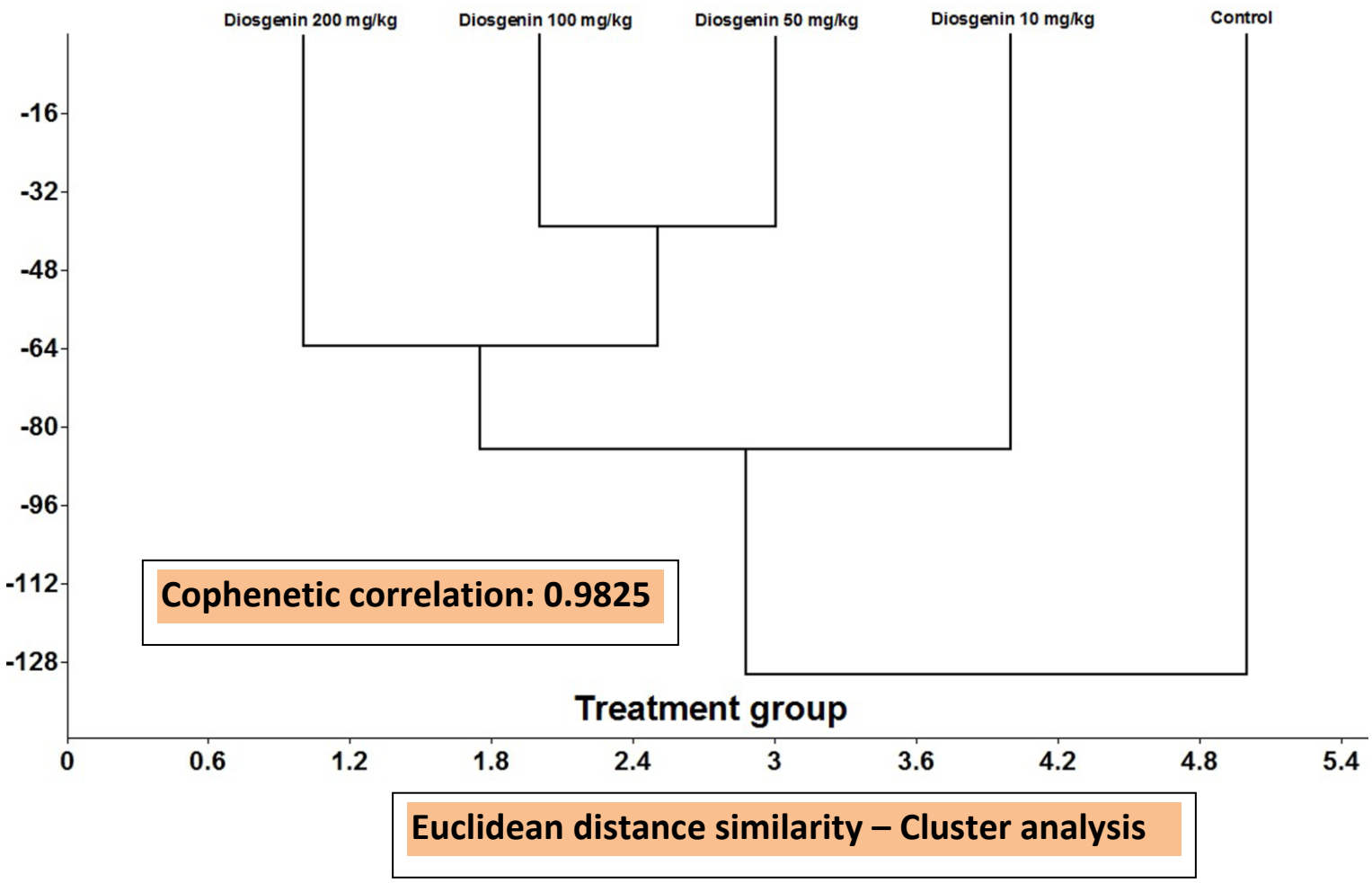

Fig. S18 Assessment of long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) on male Swiss albino mice by Euclidean distance similarity measure (cluster analysis) to catalogue the possible mode of action of diosgenin as endocrine disruptor and reproductive toxicant.

Fig. S19

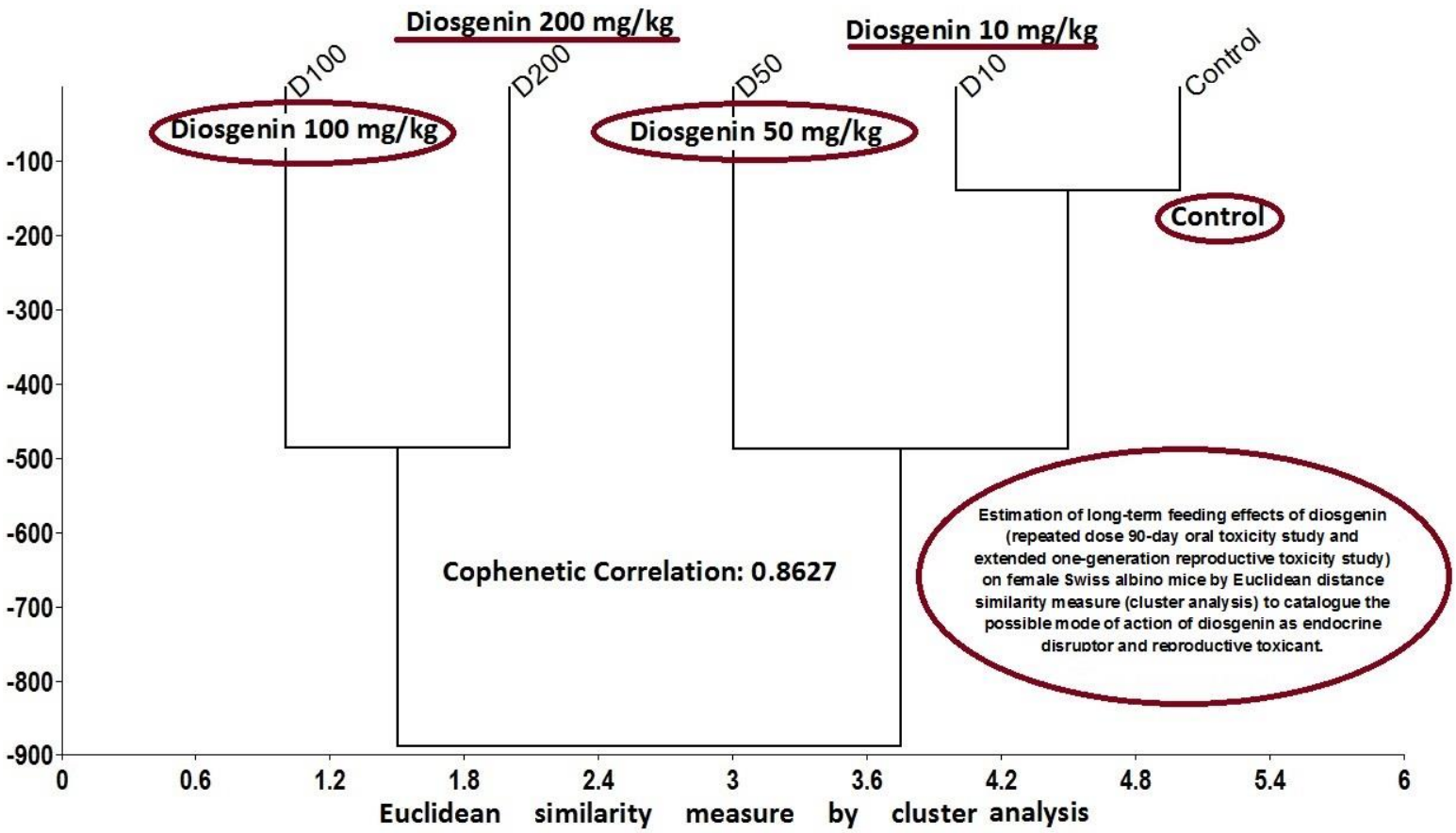

Fig. S19 Estimation of long-term feeding effects of diosgenin (repeated dose 90-day oral toxicity study) on female Swiss albino mice by Euclidean distance similarity measure (cluster analysis) to catalogue the possible mode of action of diosgenin as endocrine disruptor and reproductive toxicant.

Fig. S20

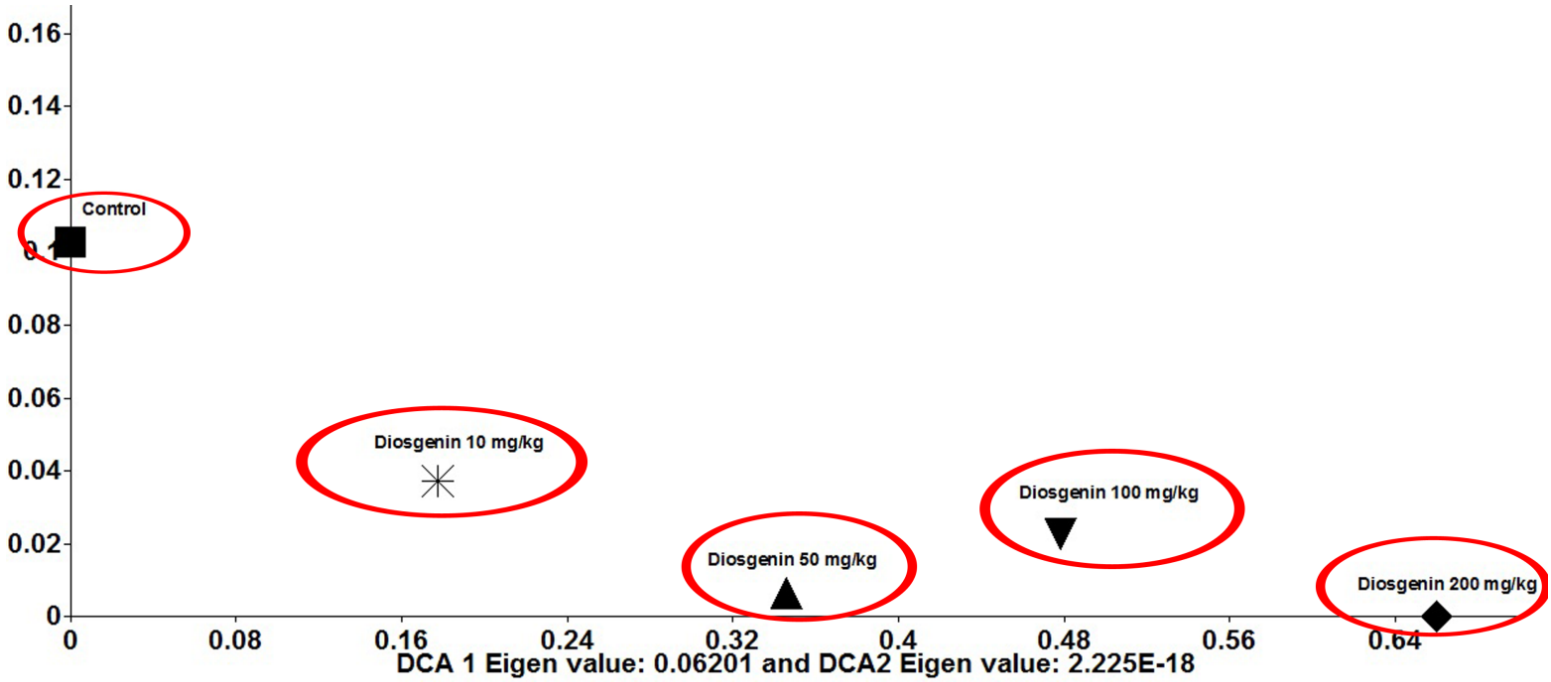

Fig. S20 Detrended correspondence analysis (DCA) splits the diosgenin treatment groups in a hierarchical fashion visualizing the endocrine disruptor and reproductive toxicant nature of diosgenin upon long-term feeding in male Swiss albino mice in repeated dose 90-day oral toxicity study.

Fig. S21

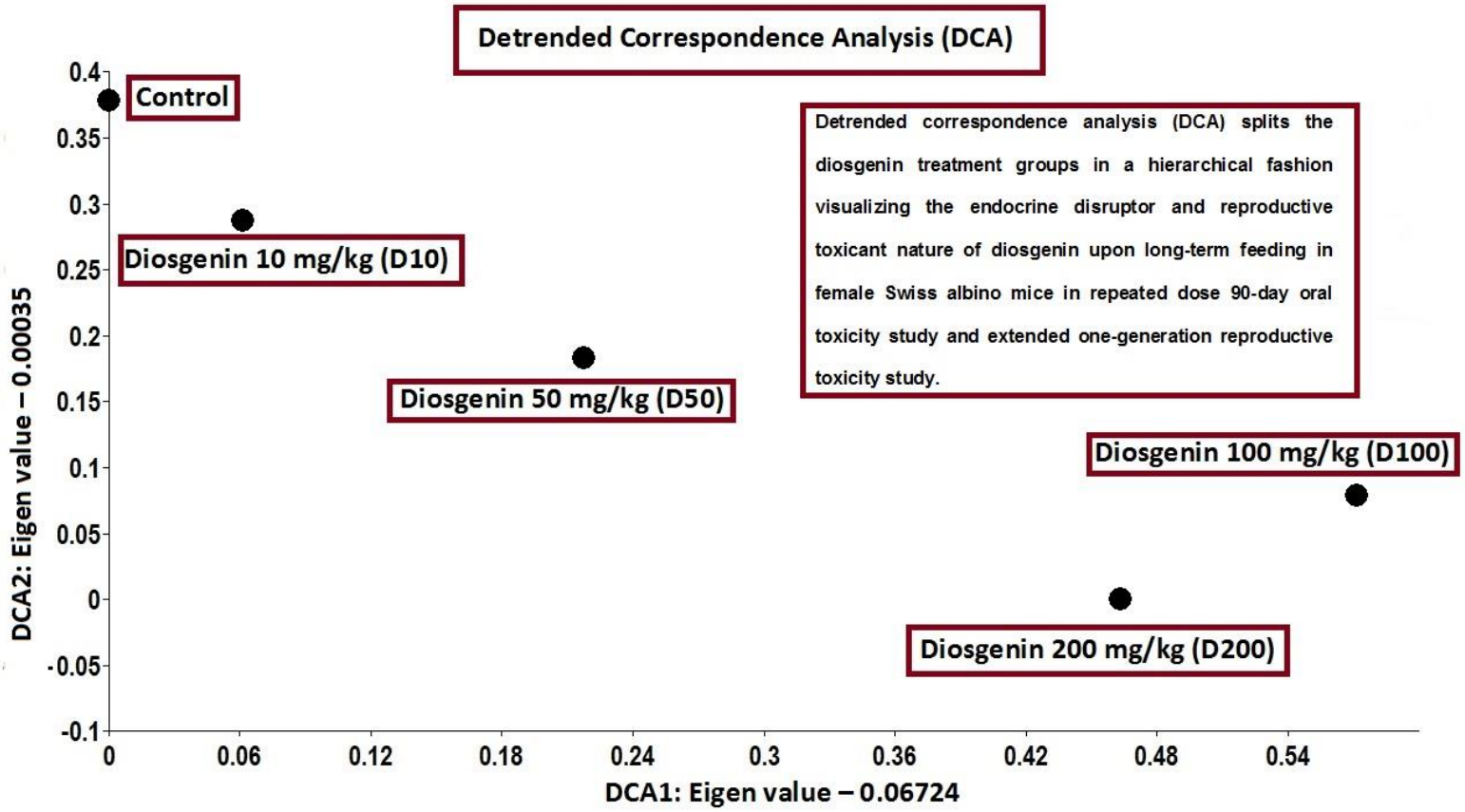

Fig. S21 Detrended correspondence analysis (DCA) splits the diosgenin treatment groups in a hierarchical fashion visualizing the endocrine disruptor and reproductive toxicant nature of diosgenin upon long-term feeding in female Swiss albino mice in repeated dose 90-day oral toxicity study.

Fig. S22

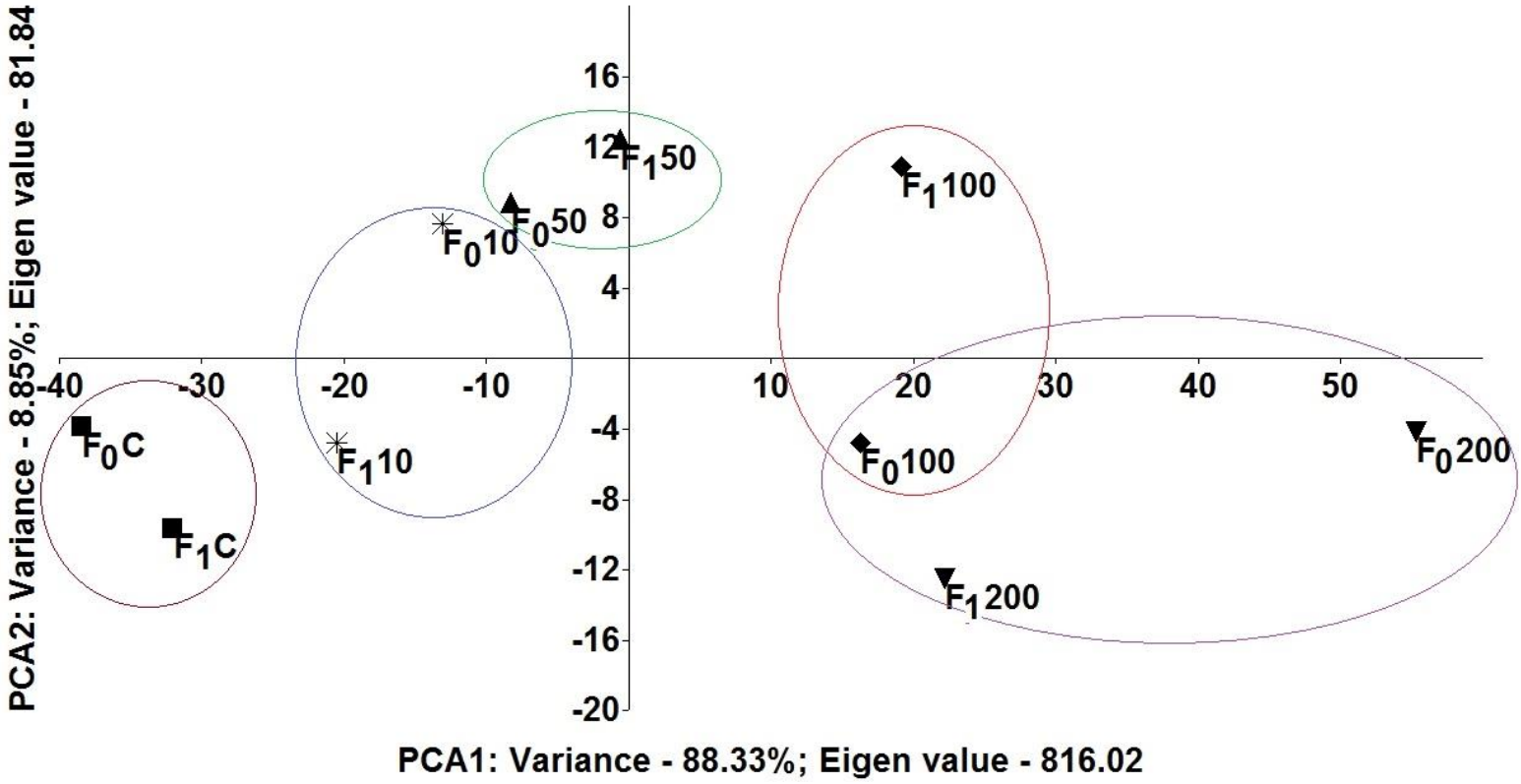

Fig. S22 Outcomes of reproductive performance, reproductive hormonal profiles and histological anomalies in F_0 and F_1 generation of male and female at postnatal day 70 (F_1 extended one-generation reproductive toxicity) showed the transgenerational endocrine disruptor and reproductive toxicant potential of diosgenin by Principal component analysis (PCA).

Fig. S23

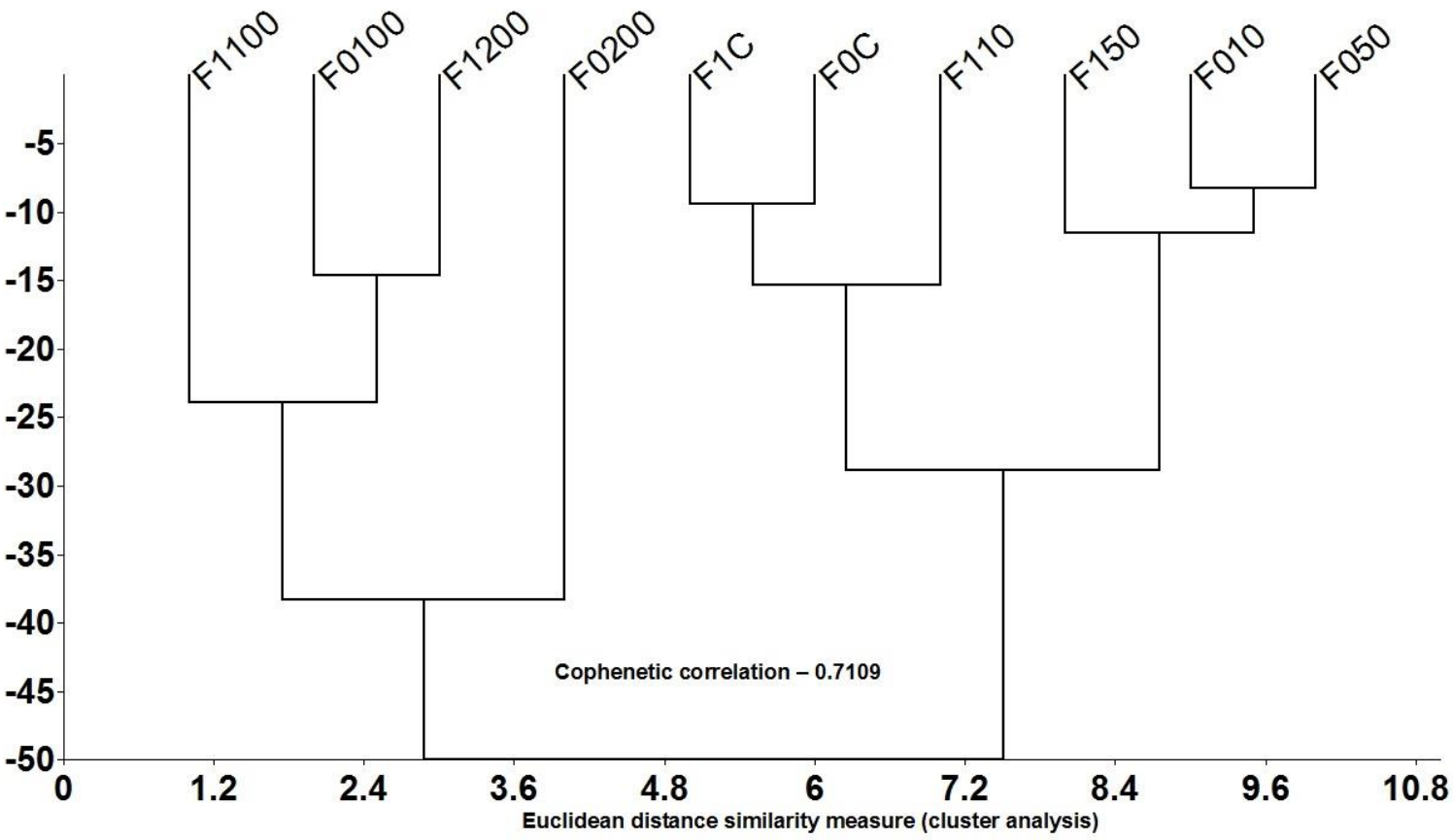

Fig. S23 Euclidean distance similarity measure (cluster analysis) categorized the transgenerational endocrine disruptor and reproductive toxicant nature of diosgenin in F_0 and F_1 generation of male and female at postnatal day 70 (F_1 extended one-generation reproductive toxicity).

Fig. S24

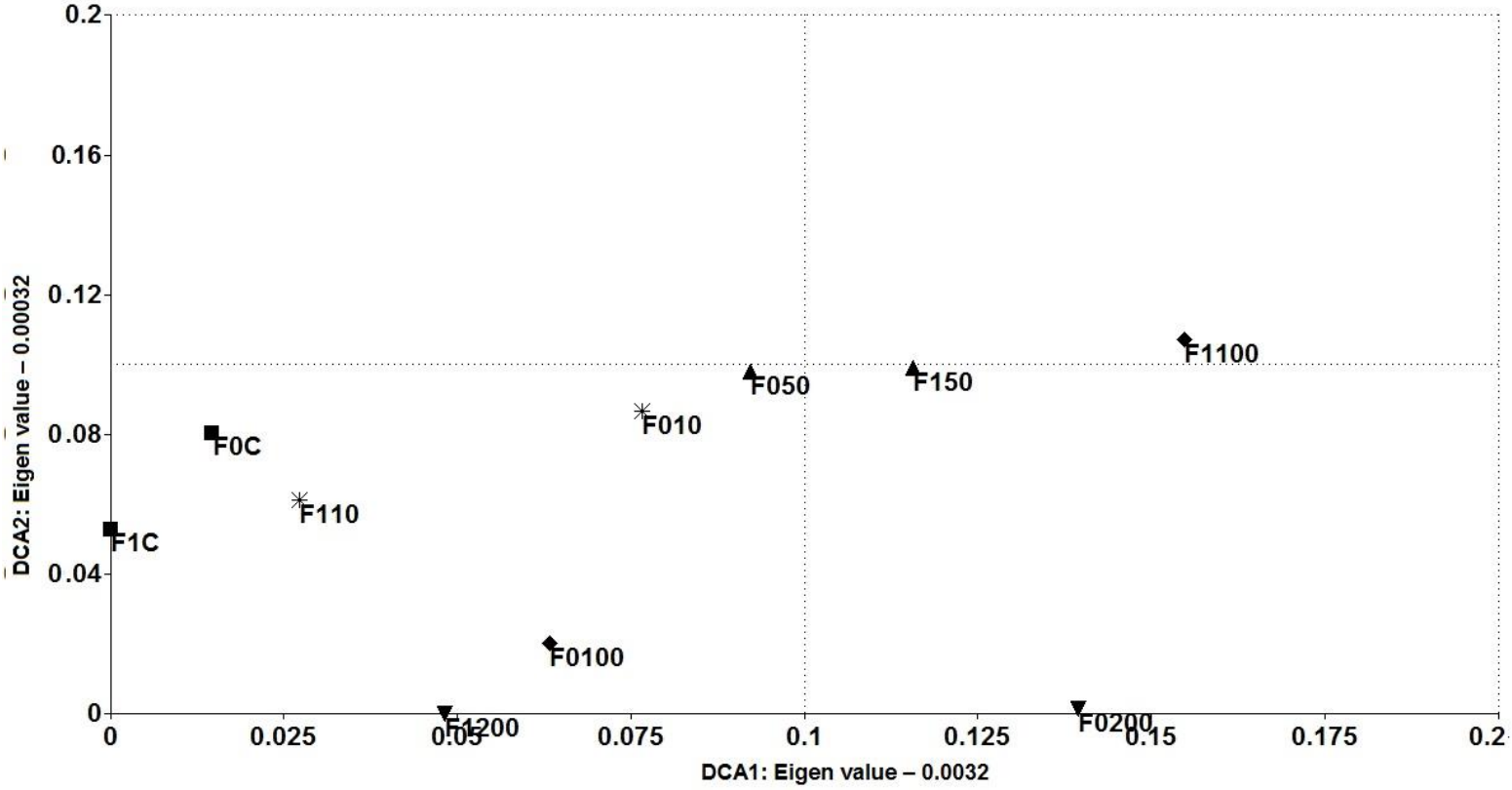

Fig. S24 Detrended correspondence analysis (DCA) discriminates the transgenerational dose dependent effects diosgenin as an endocrine disruptor and reproductive toxicant in F_0 and F_1 generation of male and female at postnatal day 70 (F_1 extended one-generation reproductive toxicity).

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Ethical Permit

26/03/2018

This is to certify that Project Title **"Diosgenin: an emerging contaminant and their possible mode of action as endocrine disruptor and reproductive toxicant"** has been scrutinized and approved by the IAEC members in compliance with the ARRIVE guidelines for the Euthanasia of animals in its meeting held on 26 March 2018. Hence the project work is permitted to undertake experiments using mice as indicated in your proposal **No. MZU/IAEC 18-19-12**. All the experiments should be strictly adhered all efforts to minimize suffering and distress of the animals. The permit number allotted for the project is **MZU-IAEC/2018/12** dated 26/03/2018.

Chairman of IAEC
26/3/18

Chairman
IAEC
Mizoram University

Member Secretary of IAEC

Member Secretary
IAEC
Mizoram University

CPCSEA Nominee