Pumpkin (*Cucurbita maxima*) seed extract ameliorates letrozole and high - fat diet - induced PCOS in rats via SIRT1/AMPK/NF - κB signaling.

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SUPPLEMENTARY DATA

Method validation for quantitative analysis of polyphenolic compounds by RP-HPLC.

The quantification of phenolic compounds was achieved from three determinations using external calibration curves constructed with five different concentrations of the corresponding standard. Limits of detection (LODs) and limits of quantification (LOQ) were calculated from the residual standard deviation (σ) of the regression curve and the slope (S), according to the following equations: LOD=3.3σ/S and LOQ=10σ/S (Table S1).

Table S1. Regression equations, LOD, and LOQ for the quantification of phenolic compounds.

Compound	Content (mg/g of PSE)	Regression equation	R^2	Range of conc. tested (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Intra-day RSD (%)	Inter-day RSD (%)
Gallic acid	18.442±1.054	y=185368x-3200.5	0.9930	0.4-2.0	0.232	0.703	3.43	2.7
Catechin	10.422±0.286	y=6642.9x+176.24	0.9908	0.4-2.0	0.266	0.805	2.44	1.74
Caffeic acid	5.746±0.169	y=39845x-275.05	0.9936	0.4-2.0	0.221	0.670	3.65	1.11
p-coumaric acid	2.368±0.001	y=3E+06x-139046	0.9965	0.4-2.0	0.047	0.142	4.12	1.49
Sinapic acid	27.141±0.971	y=133093x+961.52	0.9912	0.4-2.0	0.260	0.789	3.63	1.38
Quercetin	24.768±0.015	y=4E+06x142513	0.9918	0.4-2.0	0.251	0.759	0.24	2.52
Naringenin	8.367±0.164	y=3E+06x-30602	0.9915	0.4-2.0	0.255	0.773	0.99	1.74

Table S2. Composition of control and high-fat-diet (HFD).

Sl. No.	Ingredients	Control Diet (%)	High fat diet (%)
1	Casein	20.0	20.0
2	Cystine	0.3	0.3
3	Corn starch	45.22	7.28
4	Sucrose	17.68	17.68
5	Lodex 10 (Carbohydrate)	7.5	10.0
6	SolkaFloc (Fiber)	5.0	5.0
7	Tallow	2.0	17.75
8	Soybean oil	2.5	2.5
9	Mineral mix	5.0	5.0
10	Choline bitartrate	0.2	0.2
11	AIN-76A vitamin mix	0.1	0.1

Table S3. Sequences of primers used for Real-Time PCR

Gene		Primer Sequence 5'-3'
GAPDH	F	GGTGGTCTCCTCTGACTTCAACA
	R	GTTGCTGTAGCCAAATTCGTTGT
SIRT1	F	TGCTGGCCTAATAGAGTGGCA
	R	CTCAGCGCCATGGAAAATGT
p-AMPKα	F	TAAACCCACAGAAATCCAAACACC
	R	ACAACCTTCCATTCATAGTCCAACT
PGC-1α	F	TGAAGACGGATTGCCCTCATT
	R	GCTGGTGCCAGTAAGAGCTT
FOXO1	F	TCTAAAACTGCAAGCAGACC
	R	CCCCTAGCACTACTTACATT
SREBP-1c	F	TCAGCGAGGCGGCTTTGGAGCAG
	R	CATGTCTTCGATGTCGGTCAG
PPAR-γ	F	CTCCAATGTTCTCAAACTTAC
	R	GATGAGTCATGTAAGTTGACC
LXR-β	F	AAGCCCTGCATGCCTACGT
	R	TGCAGACGCAGTGCAAACA
NF-κB	F	GAAATTCCTGATCCAGACAAAAAC
	R	ATCACTTCAATGGCCTCTGTAG

GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase, F: Forward, R: Reverse

Table S4. Effect of low and high doses of PSE on rat organ weight.

Organ	Control	PCOS	PCOS+Met	PCOS+PSEL	PCOS+PSEH
Left ovary (mg)	$64.66\pm1.52^{b***}$	146.66 ± 2.08^{a}	67 ± 2.64^{b}	64 ± 1.0^{b}	65 ± 1.0^{b}
Right ovary (mg)	$61\pm1.0^{c***}$	148.33 ± 1.15^{a}	69.33 ± 0.57^{b}	63 ± 1.0^{c}	63.66 ± 1.15^{bc}
Liver (g)	$6.34\pm4.05^{d***}$	8.88 ± 2.08^{a}	7.33 ± 3.05^{b}	7.33 ± 1.73^{b}	6.68 ± 0.57^{c}
Uterus (g)	$5.94\pm2.08^{a***}$	3.25 ± 3.51^{e}	3.75 ± 2.08^{d}	4.52 ± 2.51^{b}	4.05 ± 4.72^{c}

Data are expressed as mean ± SD (n=6 animals in each group). Different letters (a-e) indicate significant differences among groups according to Tukey's post hoc test ($p < 0.05$). For all variables with the same letter, the difference between the means is not statistically significant. *** indicates significant difference between control and all the groups ($P<0.001$).

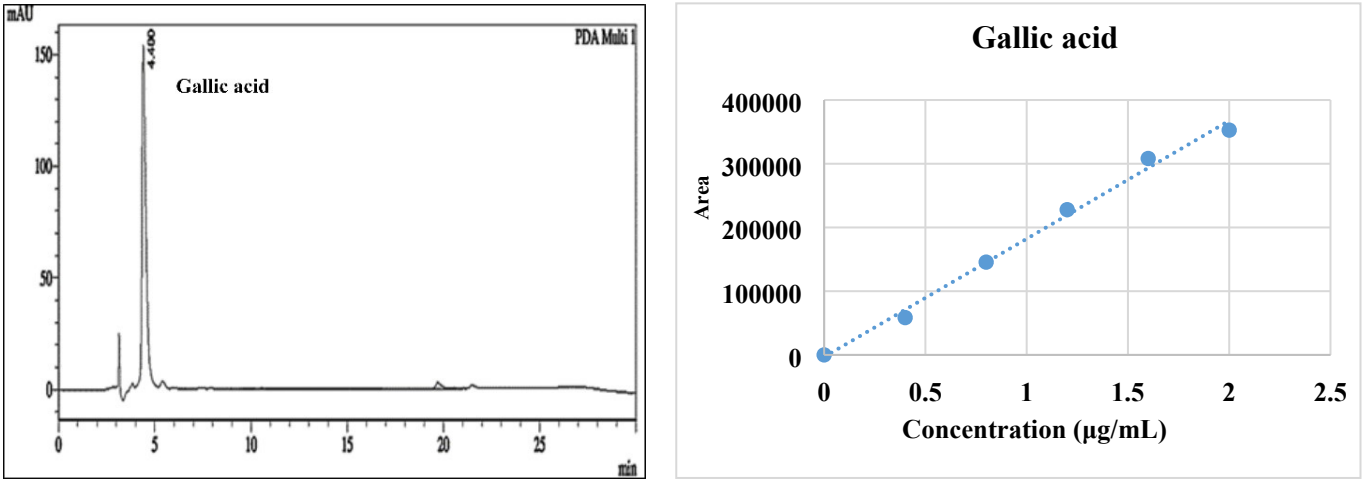
Table S5. Effect of low and high doses of PSE on rat ovary follicular count.

	Control	PCOS	PCOS+Met	PCOS+PSEL	PCOS+PSEH
Primary follicle	$6.5\pm1.17^{ab*}$	9.33 ± 0.47^{a}	4.66 ± 1.41^{b}	3.5 ± 1.17^{b}	6.83 ± 0.23^{ab}
Secondary follicle	4.16 ± 0.70^{ans}	4 ± 0.94^{a}	4.66 ± 1.41^{a}	4.83 ± 0.70^{a}	6.66 ± 2.35^{a}
Tertiary follicle	4.16 ± 2.12^{ans}	3.33 ± 0.47^{a}	8.66 ± 0.94^{a}	10.00 ± 3.77^{a}	8.00 ± 0.94^{a}
Cystic follicle	$0.0\pm0.0^{b***}$	9.5 ± 0.70^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}

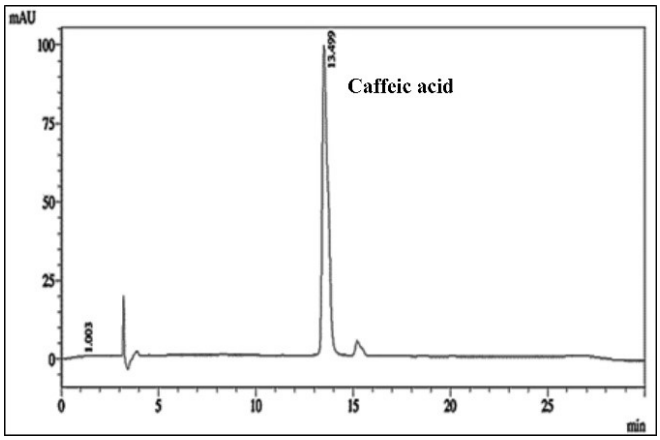
Data are expressed as mean ± SD (n=6 animals in each group). Different letters (a-e) indicate significant differences among groups according to Tukey's post hoc test ($p < 0.05$). For all variables with the same letter, the difference between the means is not statistically significant. * indicates significant difference between control and all the groups (*$P<0.05$, *** $P<0.001$, ns- Not significant).

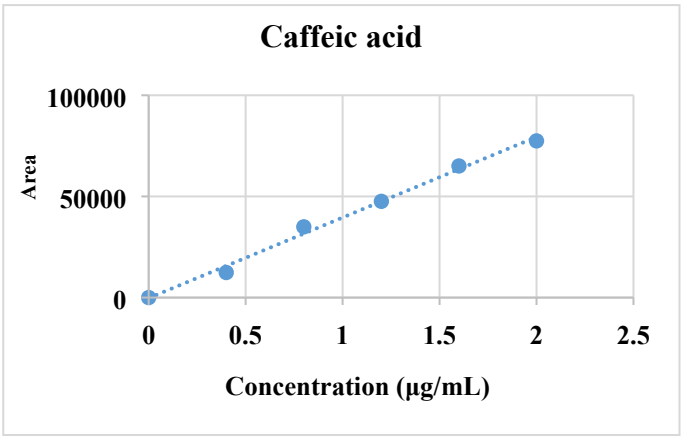
	Gallic acid	Catechin	Caffeic acid	p-coumaric acid	Sinapic acid	Quercetin	Naringenin	Total
Quantified compound (mg/g of PSE)	18.44±1.05	10.42±0.28	5.74±0.16	2.36±0.001	27.14±0.97	24.76±0.015	8.36±0.16	97.25±2.66 mg/g extract
Translated dose at 100 mg/kg of BW (PCOS + PSEL)	1.84±0.10	1.04±0.02	0.57±0.01	0.23±0.001	2.71±0.09	2.47±0.001	0.83±0.01	9.72±0.26 mg/kg of BW
Translated dose at 200 mg/kg of BW (PCOS+ PSEH)	3.68±0.21	2.08±0.05	1.14±0.03	0.47±0.003	5.42±0.19	4.95±0.003	1.67±0.03	19.45±0.53 mg/kg of BW

Table S6. Translation of RP-HPLC quantified polyphenols in PSE to *in vivo* doses.



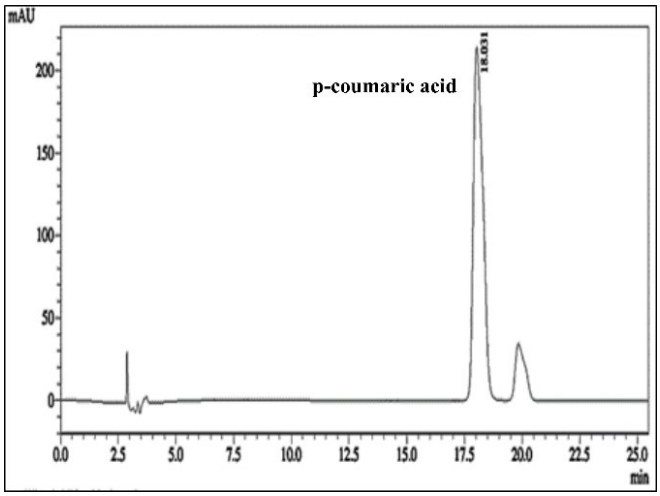
A.


mAU
Caffeic acid
13.499
1.003
min

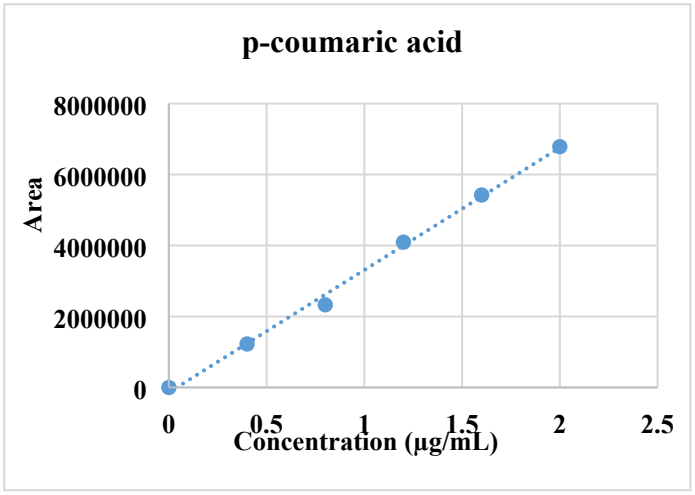

Caffeic acid
Area
Concentration (µg/mL)

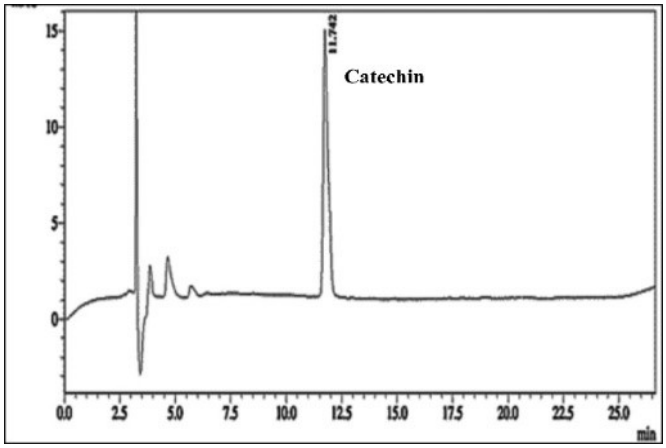
B.

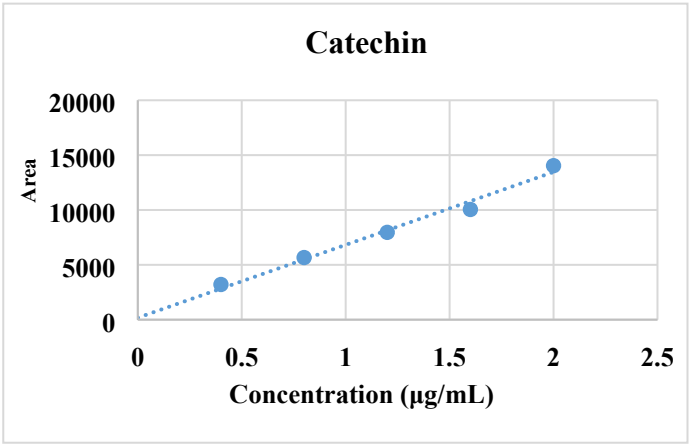
C.

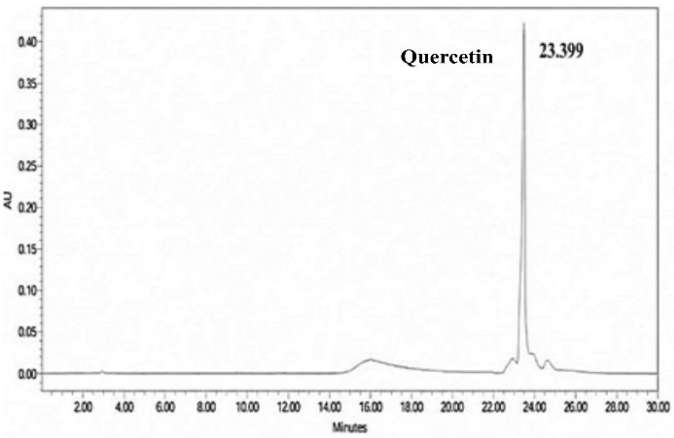

mAU
p-coumaric acid
18.031
min

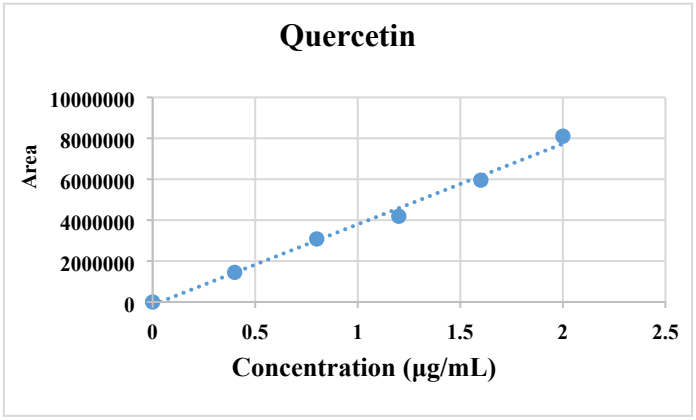
D.


p-coumaric acid
Area
Concentration (µg/mL)

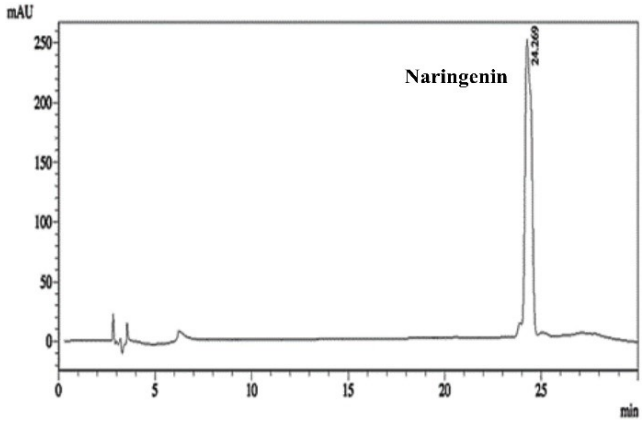

Catechin
11.742
min

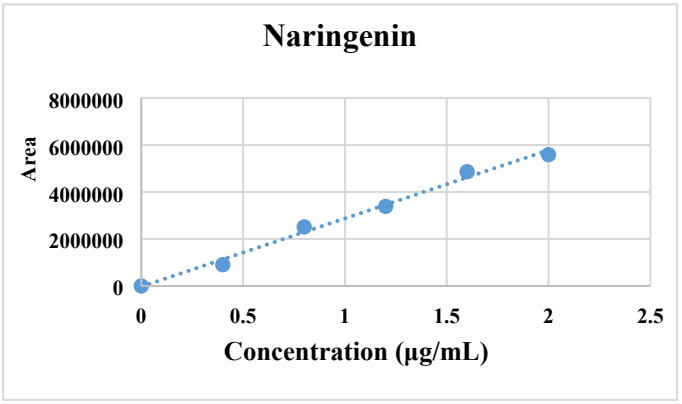

Catechin
Area
Concentration (µg/mL)





E.





F.

G.

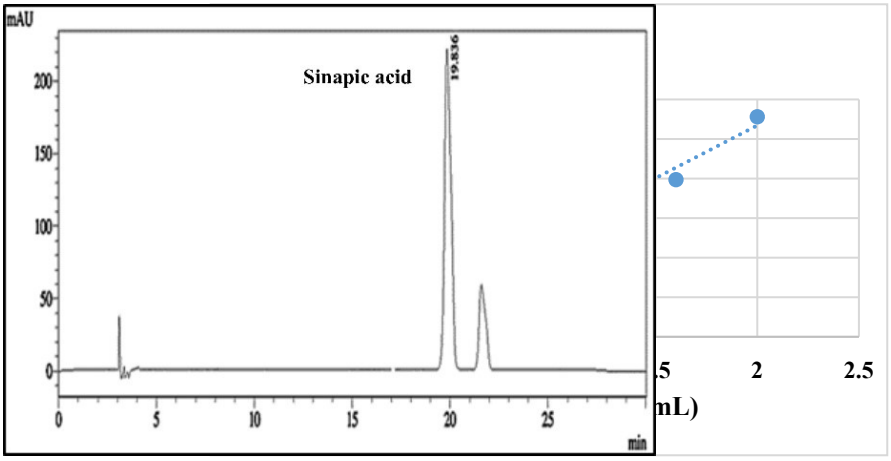


Figure S1. Representative HPLC Chromatograms (left side) and calibration curves (right side) of standard compounds. A. - Gallic acid, B. - Catechin, C. - Caffeic acid, D - p-coumaric acid, E - Sinapic acid, F - Quercetin and G - Naringenin.

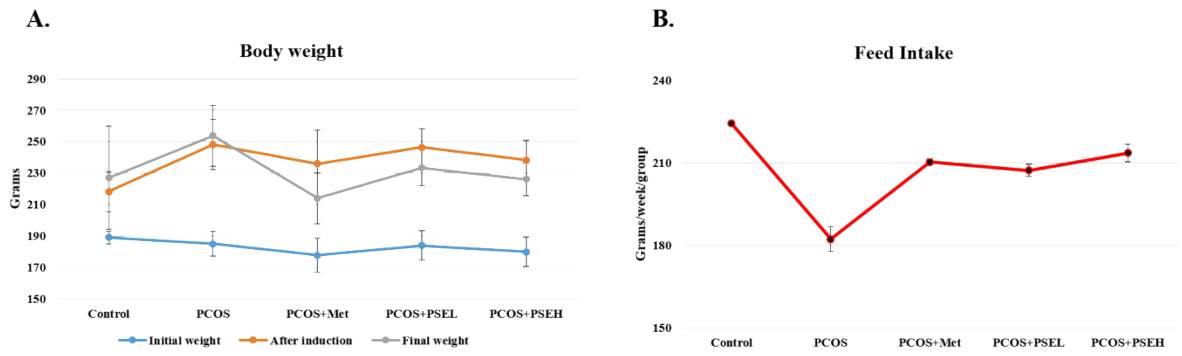


Figure S2. Effect of low and high doses of PSE on A. Animals body weight. B. Feed Intake. Data are expressed as mean ± SD (n=6 animals in each group).

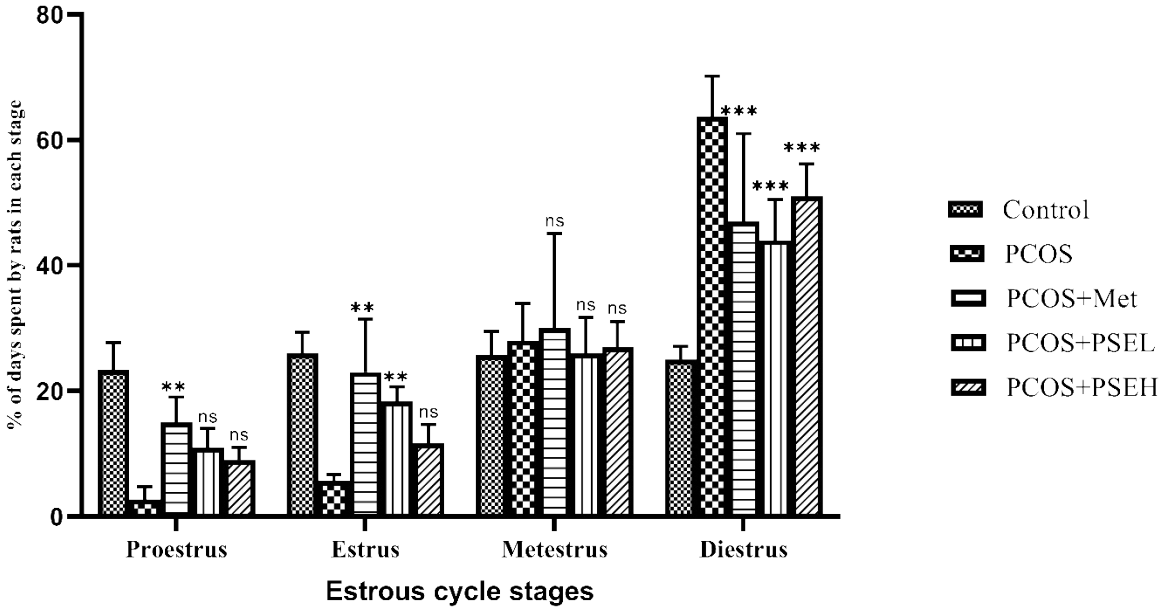


Figure S3. Percentage of days spent by the rats in each stage of the estrous cycle monitored during treatment with metformin, pumpkin seed extract, low dose and high dose (n=6 animals in each group). Different letters (a-e) indicate significant differences among groups according to Tukey's post hoc test ($p < 0.05$). For all variables with the same letter, the difference between the means is not statistically significant.); * indicates significant difference between control and all the groups (**P<0.01, *** P<0.001, ns- Not significant).

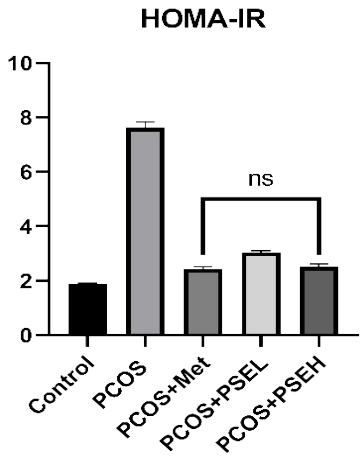


Figure S4. Effect of PSE treatment on HOMA-IR of each group. Values are expressed as mean ± SD (n=6 animals in each group). ns indicates the significant difference between PCOS+Met and PCOS+PSEH ($p>0.05$)

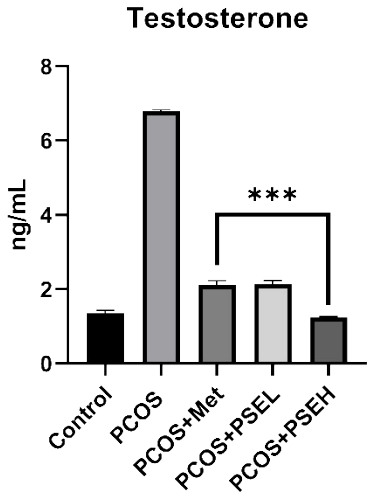


Figure S5. Effect of PSE treatment on testosterone hormone of each group. Values are expressed as mean ± SD (n=6 animals in each group). Different letters (a-e) indicate significant differences among groups according to Tukey's post hoc test ($p < 0.05$). For all variables with the same letter, the difference between the means is not statistically significant.). *** indicates the significant difference between the PCOS+Met group and PCOS+PSEH ($p<0.001$)

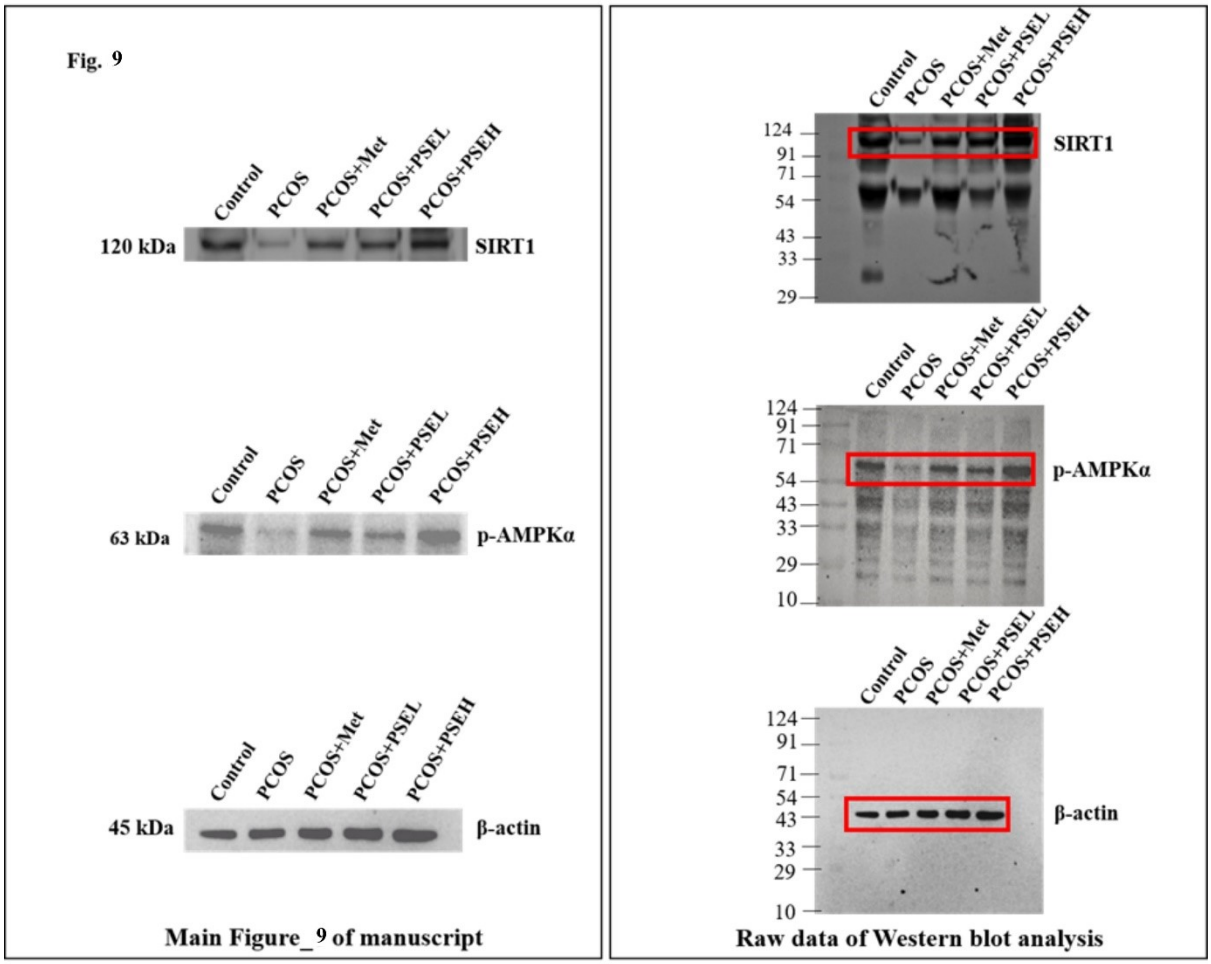


Figure S6. Full images of Western blot analysis