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SUPPLEMENTAL DATA

Astaxanthin attenuates bisphenol A–induced testicular toxicity in Wistar rats by reducing apoptosis and fibrosis via Bax/Bcl-2 balance and collagen gene expression

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Full article is available at the following link: Astaxanthin attenuates bisphenol A–induced testicular toxicity in Wistar rats by reducing apoptosis and fibrosis via Bax/Bcl-2 balance and collagen gene expression

Table S1. Statistical analysis of relative gene expression levels based on ΔCt values

One-way ANOVA Tukey's multiple comparisons test	Mean ΔCt Diff.	95% CI of Difference	Adjusted *p* value	F (DFn, DFd)	Effect size
Bax					
Control vs. Sham	0.1783	-0.4147 to 0.7714	0.8340	F (3,20) =24.64	0.7870
Control vs. BPA	1.658	1.065 to 2.251	<0.0001		
Control vs. BPA+AST	0.5633	-0.02971 to 1.156	0.0663		
Sham vs. BPA	1.480	0.8870 to 2.073	<0.0001		
Sham vs. BPA+AST	0.3850	-0.2080 to 0.9780	0.2948		
BPA vs. BPA+AST	-1.095	-1.688 to -0.5020	0.0003		
Bcl-2					
Control vs. Sham	0.02833	-0.9805 to 1.037	0.9998	F (3,20) =5.124	0.4346
Control vs. BPA	-1.183	-2.192 to -0.1745	0.0179		
Control vs. BPA+AST	-0.6217	-1.630 to 0.3871	0.3376		
Sham vs. BPA	-1.212	-2.220 to -0.2029	0.0151		
Sham vs. BPA+AST	-0.6500	-1.659 to 0.3588	0.3009		
BPA vs. BPA+AST	0.5617	-0.4471 to 1.570	0.4235		

COL1A1					
Control vs. Sham	-0.4017	-2.126 to 1.323	0.9137	F (3,20) =4.799	0.4186
Control vs. BPA	1.788	0.06365 to 3.513	0.0404		
Control vs. BPA+AST	0.6033	-1.121 to 2.328	0.7627		
Sham vs. BPA	2.190	0.4653 to 3.915	0.0099		
Sham vs. BPA+AST	1.005	-0.7197 to 2.730	0.3847		
BPA vs. BPA+AST	-1.185	-2.910 to 0.5397	0.2503		
COL3A1					
Control vs. Sham	0.01333	-0.9703 to 0.9969	>0.9999	F (3,20) =7.362	0.5248
Control vs. BPA	1.437	0.4531 to 2.420	0.0030		
Control vs. BPA+AST	0.5033	-0.4803 to 1.487	0.4949		
Sham vs. BPA	1.423	0.4397 to 2.407	0.0032		
Sham vs. BPA+AST	0.4900	-0.4936 to 1.474	0.5171		
BPA vs. BPA+AST	-0.9333	-1.917 to 0.05026	0.0667		

Data are presented as mean ΔCt differences along with their corresponding 95% CIs. Mean ΔCt Diff: This term refers to the average difference in cycle threshold values between the experimental groups. Effect Size: Reported as Eta-squared ΔCt within the overall One-way ANOVA model, this metric indicates the proportion of variance explained by the treatment. F (DFn, DFd): This notation represents the F-statistic accompanied by the degrees of freedom for treatment and residual. Adjusted *p* value:

This value is calculated using Tukey's multiple comparisons test to account for multiplicity. **Abbreviations**: ANOVA: Analysis of variance; AST: Astaxanthin; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; BPA: Bisphenol A; CIs: Confidence intervals; COL1A1: Collagen type I alpha 1 chain; COL3A1: Collagen type III alpha 1 chain; Ct: Cycle threshold; DFn: Degrees of freedom numerator; DFd: Degrees of freedom denominator; ΔCt: Delta cycle threshold.

Table S2. Statistical analysis of relative gene expression levels determined by $2^{-\Delta\Delta Ct}$ values

One-way ANOVA Tukey's multiple comparisons test	Mean $2^{-\Delta\Delta Ct}$ Diff.	95% CI of Difference	Adjusted *p* value	F (DFn, DFd)	Effect size
Bax					
Control vs. Sham	-0.1387	-1.031 to 0.7532	0.9717	F (3,20) =21.24	0.7611
Control vs. BPA	-2.244	-3.136 to -1.352	<0.0001		
Control vs. BPA+AST	-0.4858	-1.378 to 0.4062	0.4423		
Sham vs. BPA	-2.106	-2.997 to -1.214	<0.0001		
Sham vs. BPA+AST	-0.3471	-1.239 to 0.5449	0.7000		
BPA vs. BPA+AST	1.759	0.8666 to 2.650	0.0001		
Bcl-2					
Control vs. Sham	-0.009152	-0.5122 to 0.4939	>0.9999	F(3,20) = 4.639	0.4103
Control vs. BPA	0.5624	0.05934 to	0.0250		

		1.065			
Control vs. BPA+AST	0.3024	-0.2007 to 0.8055	0.3584		
Sham vs. BPA	0.5716	0.06849 to 1.075	0.0224		
Sham vs. BPA+AST	0.3116	-0.1915 to 0.8146	0.3334		
BPA vs. BPA+AST	-0.2600	-0.7631 to 0.2431	0.4866		
COL1A1					
Control vs. Sham	0.05836	-1.152 to 1.269	0.9991	F(3,20) = 12.32	0.6489
Control vs. BPA	-2.198	-3.408 to -0.9875	0.0003		
Control vs. BPA+AST	-0.2684	-1.479 to 0.9421	0.9243		
Sham vs. BPA	-2.256	-3.467 to -1.046	0.0002		
Sham vs. BPA+AST	-0.3267	-1.537 to 0.8837	0.8733		
BPA vs. BPA+AST	1.930	0.7191 to 3.140	0.0013		
COL3A1					
Control vs. Sham	0.06095	-0.8982 to 1.020	0.9979	F (3,20) =10.59	0.6137
Control vs. BPA	-1.627	-2.586 to -0.6682	0.0007		
Control vs. BPA+AST	-0.3178	-1.277 to 0.6413	0.7906		
Sham vs. BPA	-1.688	-2.647 to -0.7291	0,0004		
Sham vs. BPA+AST	-0.3787	-1.338 to 0.5804	0.6905		

BPA vs. BPA+AST	1.309	0.3504 to 2.269	0.0054		

Data are presented as mean $2^{-\Delta\Delta Ct}$ differences along with their corresponding 95% CIs. Mean $2^{-\Delta\Delta Ct}$ Diff: This denotes the average difference in cycle threshold values between the experimental groups being compared. Effect Size: Reported as Eta-squared 2-ΔΔCtfor the overall one-way ANOVA model, this indicates the proportion of variance explained by the treatment. F (DFn, DFd): This represents the F-statistic, including degrees of freedom for both treatment and residuals. Adjusted *p* value: This value is calculated using Tukey's multiple comparisons test to adjust for multiplicity.

Abbreviations: ANOVA: Analysis of variance; AST: Astaxanthin; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; BPA: Bisphenol A; CIs: Confidence intervals; Ct: Cycle threshold; DFn: Degrees of freedom numerator; DFd: Degrees of freedom denominator.

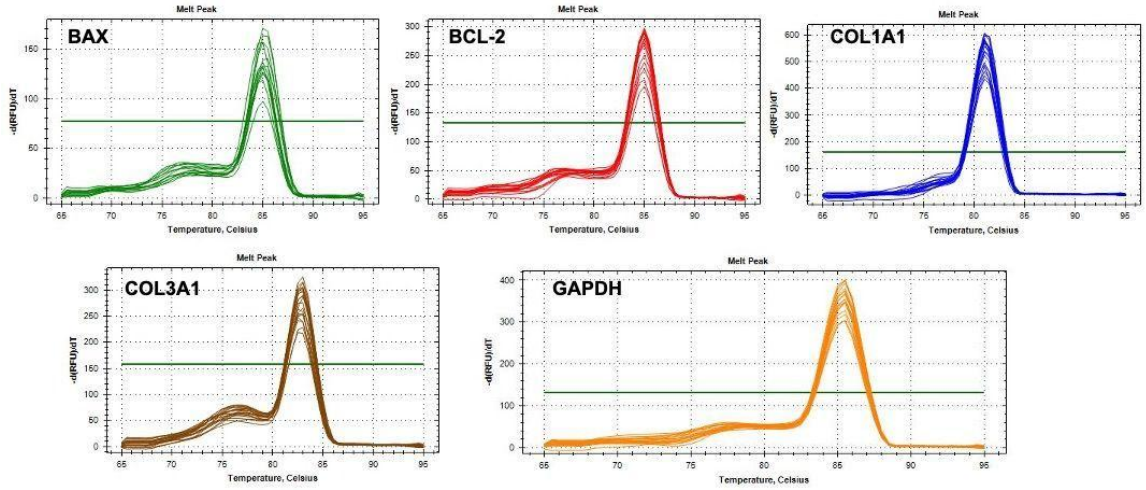


Figure S1. Melt curve analysis for primer validation. Melt curve profiles (derivative fluorescence vs. temperature) are shown for Bax, Bcl-2, COL1A1, COL3A1, and GAPDH RT-qPCR assays. For each primer pair, the overlaid traces from all samples display a single, sharp melt peak, supporting specific amplification of a single product and arguing against substantial primer–dimer or non-specific amplicon formation. Negative controls (NTC and No-RT) were included during assay validation to exclude contamination and genomic DNA–derived signals (not shown).
Abbreviations: Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; COL1A1: Collagen type I alpha 1 chain; COL3A1: Collagen type III alpha 1 chain; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; No-RT: No reverse transcription; NTC: No template control; RT-qPCR: Reverse transcription quantitative polymerase chain reaction.