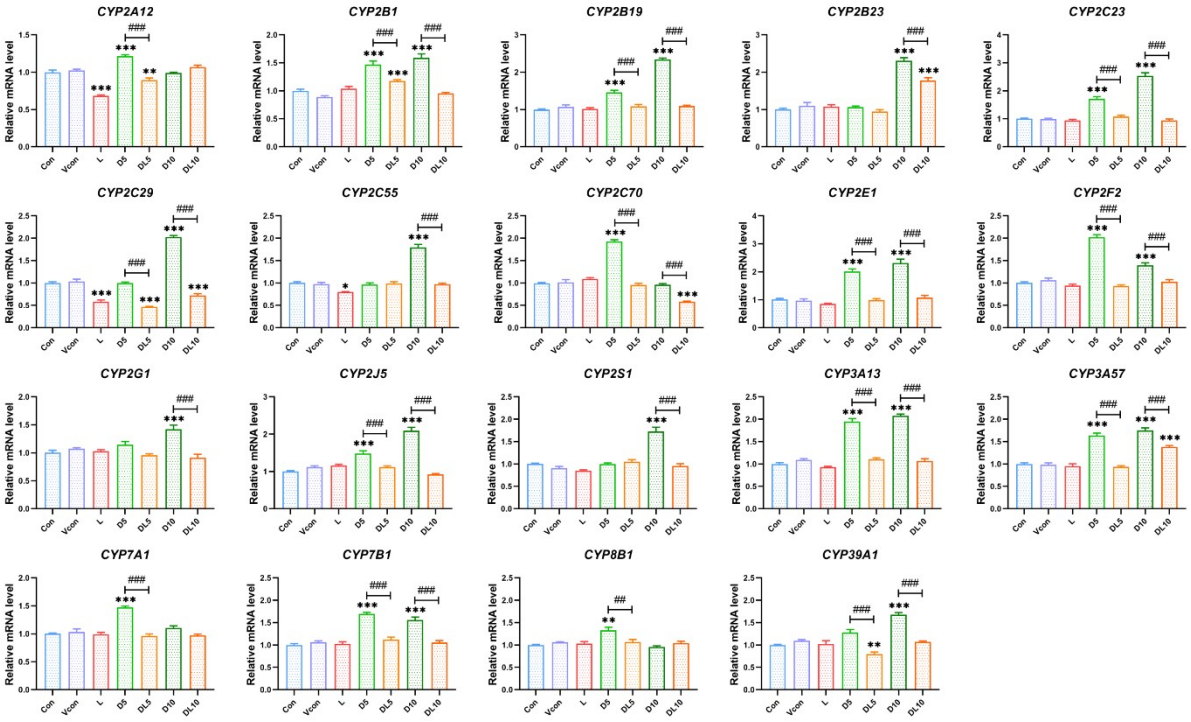
Electronic Supplementary Material (ESI) for Food & Function.

Supplemental Materials

Fig. S1. The NXR signaling pathway related genes mRNA expression of testis



Data are presented as the mean ± SEM. Symbol for the significance of differences between the vehicle control group and another group: **P* < 0.05, ***P* < 0.01, ****P* <0.001. Symbol for the significance of differences between the DEHP-treated groups and the DEHP + 5 mg/kg LYC treatment group: $^{\#\#}P$ < 0.01, $^{\#\#\#}P$ <0.001.

Tab. S1. Animal groups*

Group	N	LYC concentration (mg/kg BW[6] /day)	DEHP concentration (mg/kg BW[6] /day)
Con[1]	20	0	0
Vcon[2]	20	0	0
L[3]	20	5	0
D5[4]	20	0	500
DL5[5]	20	5	500
D10[4]	20	0	1000
DL10[5]	20	5	1000

* Corn oil as vehicle dissolves DEHP and LYC.

[1] Con: treatment control (distilled water).

[2] Vcon: vehicle control (Corn oil).

[3] L: antagonistic control.

[4] D5 and D10: DEHP-exposed groups.

[5] DL5 and DL10: LYC antagonized DEHP-exposed groups.

[6] BW: Body Weight

Tab. S2. Sequences of oligonucleotide primers for QRT-PCR

Gene Names	Sequence (5' → 3')	NCBI Reference Sequence	Amplicon size (bp)
GADPH	AAGGTCGGTGTGAACGGATT CAACAATCTCCACTTTGCCACT	NM_001289726.1	82
β-actin	CAAGAGAGGTATCCTGACCT TGATCTGGGTCATCTTTTCAC	NM_007393.5	188
CAR	CCCATCTGTCCGTTTGCT TTCCTCATGCCAACATTTAGACAC	NM_009803.5	101
PXR	GTGTCTTCCGGGTGATCTCG GGTCTTCAAATCTGCCGTGTATG	NM_010936.3	163
CYP2A12	TGAGACAGTCAGCTCCACAC CCTCATGGACCTTGGCCTCT	NM_133657.1	80
CYP2B10	AGCCAACCTTCAAGGAATATGGTG ATTGGGCTTCCTCCTGAATCCG	NM_009999.4	141
CYP2B19	TGCCCGCAATGAATTGTTCC TTGCACCACTGTTATTGGGA	NM_007814.2	110
CYP2B23	GATTCAGCCAATTGTACAGGAC ACTCCGCTTTCCCATCCCAA	NM_001081148.1	115
CYP2C23	ATTCGGGCTTCTGCTCCTTG GGGCAGCTTCATCTTGTCCT	NM_001001446.3	118
CYP2C29	CATGGAGCTGTTTCTAATCCTG GAGGCAAATCCATTCATCACT	NM_007815.3	108
CYP2C55	TGCAGGTCTACAATGCTCT GTTCTTTCACTCGCCCCAAA	NM_028089.3	114
CYP2C70	CCACAGTGAAATATGGGCTT GGGTCTCCGATGTCTACCAA	NM_145499.2	101
CYP2E1	CACAGCCAAGAACCCATGTACA CAGGAGCCCATATCTCAGAGTTG	NM_021282.3	108
CYP2F2	CCTTTGACCCCGTGTTTATCC TCGAAGCGACTTCCGAAGAC	NM_007817.2	76
CYP2G1	CCAAATACTTCCGCTACCCAGA GACACAGATGCGCTTTCCAG	NM_013809.1	116
CYP2J5	GAAATCCAAGAAAATGTACAGGC CATGGACTCTCGGTCAGACA	NM_010007.4	84

CYP2S1	TCCCTCATCTTTGGCATCCG ATCCCCAACAAGGTCCCACT	NM_028775.4	86
CYP3A13	CTGCCACCTATGATACCCTG CTCTCAAGTCTTCCAGCGAT	NM_007819.4	94
CYP3A57	TGGACCTAAACCACTGCCTT TGTCGTCCCTCATAAAACCCC	NM_001100180.1	120
CYP7A1	CGGGAAAGCAAAGACCACCT TATTGTCGCGCCTGATCCGAA	NM_007824.3	82
CYP7B1	CCGATTCTGCCGTCTCCTT GCAGCCTTACTCTGCAAAGCTT	NM_007825.4	85
CYP8B1	TTCCCCAGGTTTGTCTACTCC AACCAACAGCTTATGCCGTCT	NM_010012.3	128
CYP39A1	CATGAGCGACTGTATGCCTT TTCCATGAGTGCCTAAACCT	NM_018887.4	118
RXR	GTCCACCAGCAGTGCCAACGA CCCCATGTTTGCCTCCACGTA	NM_011305.3	103
ATF4	ACATTCTTGCAGCCTTTCCC TCAACTTCACTGCCTAGCTCT	NM_009716.3	89
ATF6	ATTACCAGCTACCACCCACA AGTCCTGCCCATTGATCACA	NM_001081304.1	98
GRP78	CCAAAACCGCCTGACACCT ATTCCTGGTGTCAATGCGCTCT	NM_001163434.1	103
GRP94	CAAGCATACCAGACGGGCAA CTTCCTTAATCCGCCGCAAC	NM_011631.1	118
CHOP	CTCGCTCTCCAGATTCCAGT TGACCACTCTGTTTCCGTTT	NM_007837.4	91
EIF2a	CAGCTTATAGACCTCCAGCCTT ATGGCTTGTCACTTCCTGGAT	NM_001005509.2	108
PERK	ATCAGCACTTTAGATGGACGAA AGACCCCACGTCCAAATCCCA	NM_001313918.1	81

Supplementary 1. Details of Materials and Methods

Supplementary 1.1 Determination of CYP450 content and activities

CYP450: The total CYP450 content was detected by measuring the difference spectrum of dithionite-reduced CO. The reaction mixtures contained 0.3 mL testis suspension and 5.7 mL phosphate buffer (PBS). After 2 min, the reaction solution was divided into two parts and added into two 3 mL cuvettes for scanning. Then the samples were inserted into CO and the absorption was measured with an ultraviolet–visible spectrophotometer. Activity was calculated using an extinction coefficient of 91 mM^{-1} cm^{-1} (450–490 nm) and expressed in nmol mg^{-1} of protein.

NADPH-cytochrome c reductase (NCR): The reaction mixtures contained 0.2 mL testis suspension, 2.8 mL PBS and 0.1 mL cytochrome C were divided into two cuvettes. 0.02 mL NADPH (5 mg mL^{-1}) and the same volume of phosphate buffer were added into sample cell and reference cell, respectively. The absorption continuously recorded once every 1 min for 3 min at 550 nm by spectrophotometer. The results were expressed as nmol mg^{-1} of protein using an extinction coefficient of 19.1 mM^{-1} cm^{-1}.

Aminopyrine N-demethylase (APND) and erythromycin N-demethylase (ERND) activity: APND and ERND activity were measured by the formation of formaldehyde. The mixture of testis suspension (0.5 mL), aminopyrine/erythromycin (0.1 mL) and potassium phosphate (0.4 mL) were incubated at 37 °C for 3 min. The reaction was initiated by adding NADPH (0.1 mL), incubated for 30 min at 37 °C. The absorbance was recorded at 412 nm with an ultraviolet–visible spectrophotometer. The results were expressed as nmol of HCHO min^{-1} mg^{-1} of protein.

Aniline-4-hydeoxylase (AH): AH was quantified by the NADPH-dependent formation of 4-aminophenol. 1 mL NADPH (1.0mmol/L) and the same volume of PBS were added into sample cell with 0.5 mL aniline hydrochloride and reference cell for 2 min at 37 °C. The 0.5 mL of testis suspension was added to each tube

for 30 min at 37 °C, and then was added 1 mL ice-cold TCA (200 mL/L) to stop the enzyme reaction. The reaction mixtures were ice bath for 5 min and centrifuged at 11,000g for 10 min. Then the reaction mixtures were taken 1mL supernatant in another test tube, added 1 mL 4-aminophenol (10 mL/L) and then added 1 mL sodium carbonate (1 mol/L) for 30 minutes. The absorption recorded at 630 nm by spectrophotometer. The results were expressed as nmol mg^{-1} of protein

7-ethoxyresorufin-O-deethylase (EROD): The 2ml reaction solution includes 0.1 M Tris-HCl buffer (pH 7.8), 150 mM KCl, 0.1 mM EDTA, 5 mM $MgCl_2$, 0.1 mg testis suspension and 10 µL of 0.5 mM 7-ethoxyresorufin. The reaction mixtures were added 10 µl of 0.01 M NADPH to start the reaction. The emission wavelength is 380nm, the excitation wavelength is 460nm, respectively.

Supplementary 1.2 Quantitative real time PCR (qPCR)

The testis tissues were stored in RNA locker (Beijing Tiandi, China) and frozen at −80 °C. RNA out reagent (Beijing Tiandi, Inc. China) was used to extract total RNA from the testis (50 mg tissue) from each group. The total RNA was quantitated using the spectrophotometer (GE, USA) and purity was assessed from the 260/280 nm ratio. After synthesizing the single-stranded cDNAs, qPCR was performed to quantify gene expression in the LineGene 9620 (Hangzhou Bioer Technology Co., Ltd., China). Relative RNA equivalents for sample were obtained by standardization of GAPDH and β-actin. Relative transcript abundance was calculated using the $2^{-\Delta\Delta CT}$ method. Gene primer sets are given in Table S1.

Supplementary 1.3 Western Blotting

The testis tissue samples were cracked in cold RIPA lysis buffer (Beyotime, Shanghai, China) with the protease inhibitor cocktail (MedChem Express, HY-K0010, USA). Then, the samples were pelleted by centrifugation at 12000 rpm for 15 min at 4 °C and boiled in 5× SDS-PAGE sample loading buffer (Beyotime, P0015, Shanghai, China). The Proteins were separated on 12% SDS-PAGE gels, transferred to NC

membranes, and immunoblotted with antibodies ABclonal Technology (Wuhan, Hubei, China), Bioss (Beijing, China) and ZSGB-BIO (Beijing, China). The bands of protein were exposed by using Amersham Imager 600 (GE, Switzerland) and quantified by use of ImageJ software (National Institutes of Health, USA).

Supplementary 1.4 Immunohistochemistry (IHC)

For IHC, sections were blocked with superblock buffer (Pierce, Rockford, IL) for 30 min. The sections were then incubated with primary antibodies overnight at 4 °C. After sections were washed with PBS and incubated with secondary antibodies for 2 h at room temperature. For the development of color, sections were treated with diaminobenzidine for 2 min and counterstained with hematoxylin.25 The final images were captured by using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached photographic machine (BX-FM; Olympus Corp, Tokyo, Japan). The positive area was quantified by the Image Pro Plus software.