

Supplementary Material

Lipidomic profiling of subchronic As₄S₄ overexposure identifies inflammatory mediators as sensitive biomarkers in rats

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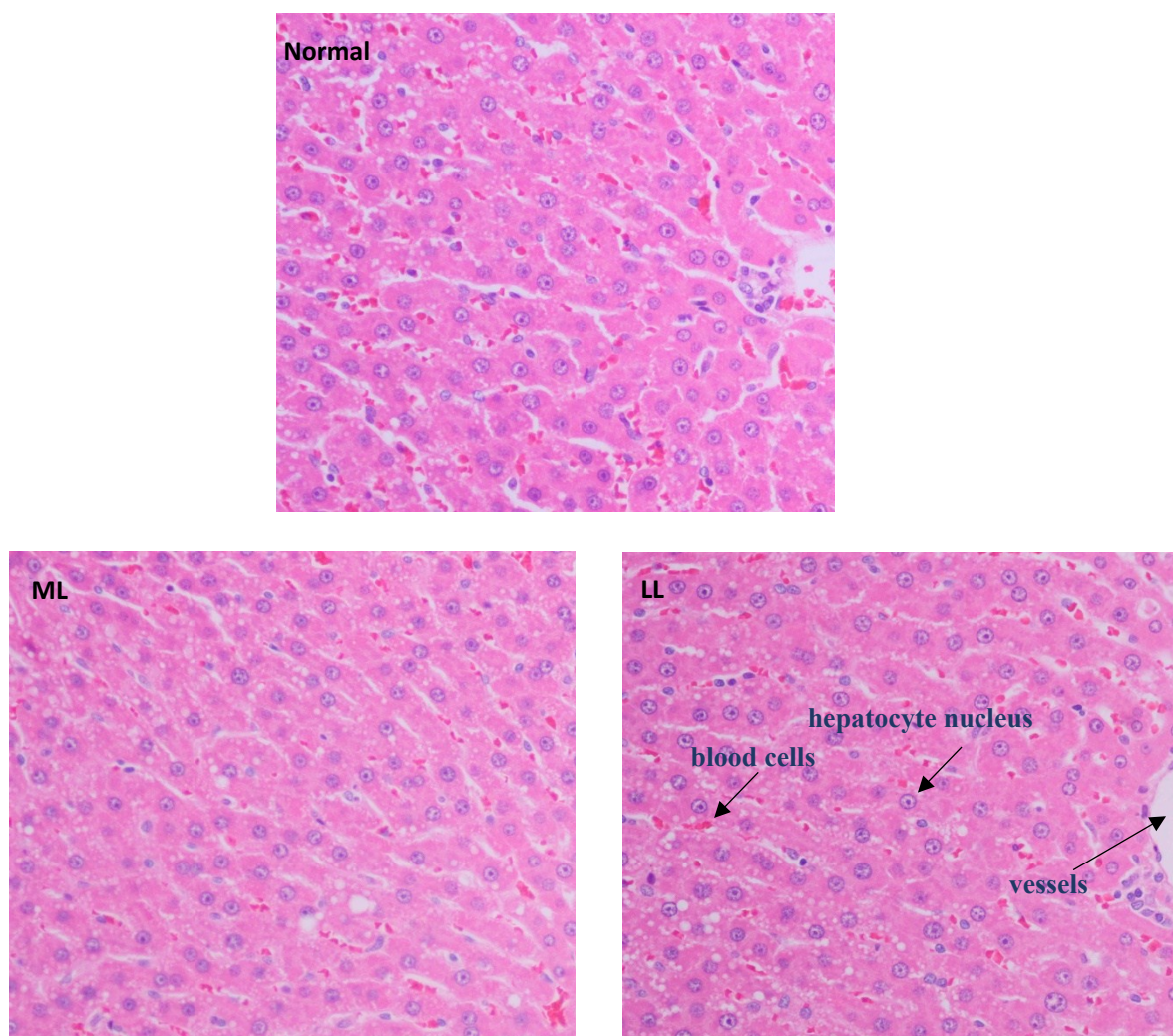
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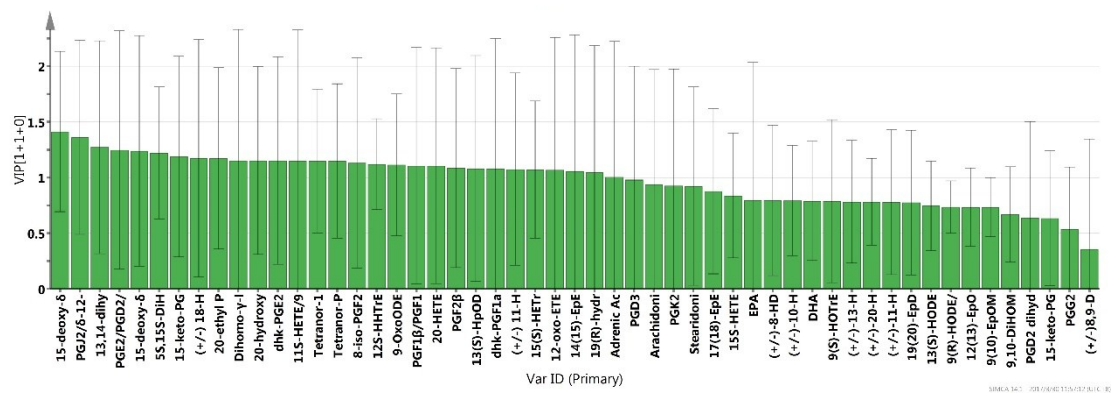
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Supplementary Figure 1. Histopathology of the liver in rats after arsenic exposure from As₄S₄ for 2 months. Normal group; ML: 41 mg/kg As₄S₄ group; MH: 134 mg/kg As₄S₄ group (magnification: ×100).



Supplementary Figure 2. Multivariate analytical results from lipidomics profiles in normal and As₄S₄ groups. VIP of OPLSDA model for As₄S₄ (41 mg/kg) vs. control group.

Supplementary Table 1. Levels of liver function indexes in liver of rats following oral administration of As₄S₄ for two months.

groups	ALT (U/L)	AST (U/L)	TBIL (μmol/L)	ALP (U/L)	ALB (g/L)	LDH (U/L)
N	33.43±5.85	122.99±22.66	1.85±0.68	72.7±22.16	29.74±1.21	246.3±45.29
ML	26.3±5.59	106.23±22.92	1.46±0.59	55.1±8.92	29.72±1.17	222.8±77.53
MH	26.9±2.79	95.47±19.26	1.94±0.88	61±11.91	30.25±0.81	198.5±31.07

Nor: Normal group; ML: 41 mg/kg As₄S₄ group; MH: 134 mg/kg As₄S₄ group. Values were expressed as mean ± SD (n=10).

Supplementary Table 2. MRM transitions for the LC-MS/MS assay of lipids

Abbreviation	MS	MS/	DP	CE	Time	Abbreviation Name	MS	MS/	DP	CE	Time
Name		MS			(min)			MS			(min)
Tetranor-12-HETE	265.2	97	-75	-14	14.1	11-HDoHE	343.2	133	-60	-19	9.0
Stearidonic Acid	275.2	231	-60	-30	6.9	10-HDoHE	343.2	161	-60	-17	9.0
12-HHTrE	279.2	97	-30	-30	16.6	16-HDoHE	343.2	189	-70	-59	8.5
9-oxoODE	293.2	125	-60	-25	8.8	16,17 EpDPE	343.2	189	-70	-59	10.0
13-oxoODE	293.2	249	-70	-30	8.4	13-HDoHE	343.2	193	-60	-17	8.9
9-HOTrE/13-HOTrE	293.2	275	-70	-20	7.0	14-HDoHE	343.2	205	-60	-30	8.8
12,13 EpOME	295.0	195	-60	-25	9.8	20-HDoHE	343.2	281	-60	-20	8.1
9-HODE	295.2	171	-60	-25	8.3	17-HDoHE	343.2	281	-60	-20	8.4
9,10 EpOME	295.2	171	-60	-25	9.9	7-HDoHE	343.2	281	-60	-20	9.0
13-HODE	295.2	195	-60	-25	8.2	4-HDoHE	343.2	281	-60	-20	9.9
EPA	301.2	203	-60	-30	12.8	LXA ₅	349.0	113	-60	-25	3.0
Arachidonic acid	303.2	59	-60	-30	14.0	Resolvin-E ₁	349.2	161	-60	-30	2.7
Dihomo- γ -linolenic acid	305.2	287	-60	-30	14.7	PGD ₃	349.2	189	-55	-25	2.8
13-HpODE	311.2	293	-60	-30	8.3	PGK ₂	349.2	205	-50	-30	14.0
9,10-diHOME	313.2	201	-60	-30	6.1	PGE ₃	349.2	262	-55	-25	2.8
15d-PGJ ₂	315.2	271	-50	-15	6.9	15k PGE ₂	349.2	315	-35	-30	5.1
15d-PGA ₂	315.2	271	-50	-15	7.5	15k PGF _{2α}	351.2	217	-41	-35	3.4
14,15 EpETE	317.0	208	-65	-18	7.8	6-LXA ₄	351.2	115	-30	-20	3.7
17,18 EpETE	317.0	215	-65	-16	8.4	20oh LTB ₄	351.2	151	-60	-25	3.0
11-HEPE	317.2	121	-60	-30	7.6	dhk PGD ₂	351.2	175	-40	-25	3.9

8-HEPE	317.2	161	-70	-25	7.7	PGF _{3α}	351.2	193	-75	-30	3.4
5-oxoETE	317.2	163	-40	-25	9.8	PGE ₂	351.2	271	-50	-25	3.4
12-HEPE	317.2	179	-40	-25	7.8	PGD ₂	351.2	271	-50	-25	3.5
12-oxoETE	317.2	179	-40	-25	8.8	dhk PGE ₂	351.2	333	-40	-25	3.4
15d-PGA ₁	317.2	219	-60	-30	7.6	PGF _{2α}	353.2	193	-50	-30	3.2
18-HEPE	317.2	255	-60	-20	6.9	dhk PGF _{2α}	353.2	209	-60	-25	3.2
15-HEPE	317.2	255	-60	-20	7.1	dhk PGE ₁	353.2	221	-60	-30	4.7
9-HEPE	317.2	255	-60	-20	7.6	PGE ₁	353.2	223	-55	-25	4.0
5-HEPE	317.2	255	-60	-20	8.1	PGD ₁	353.2	235	-60	-30	4.0
16-HETE	319.0	189	-80	-25	7.9	8-iso-PGF _{2α}	353.2	309	-50	-30	3.2
19-HETE	319.0	231	-80	-25	7.5	11β-PGF _{2α}	353.2	309	-50	-30	3.2
17-HETE	319.0	247	-80	-25	8.0	dh PGE ₁	355.2	237	-60	-30	5.0
11-HETE	319.2	167	-60	-25	8.9	PGF _{1α}	355.2	311	-60	-30	14.3
9-HETE	319.2	167	-60	-25	9.5	dh PGF _{2α}	355.2	311	-60	-30	15.0
15-HETE	319.2	219	-50	-15	8.6	dhk PGF _{1α}	355.2	337	-70	-22	15.0
12-HETE	319.2	257	-60	-20	8.6	15t PD ₁	359.0	153	-40	-20	5.5
8-HETE	319.2	257	-60	-20	9.1	10,17-diHoHE	359.0	153	-40	-20	5.8
5-HETE	319.2	257	-60	-20	9.5	PD ₁	359.2	153	-40	-20	5.4
20-HETE	319.2	301	-75	-25	7.6	19,20-diHDP A	361.0	229	-70	-25	6.4
15-oxoEDE	321.0	113	-100	-32	10.8	dihomo PGJ ₂	361.0	299	-55	-25	4.5
5-HETrE	321.2	259	-70	-19	9.6	dihomo 15d PGJ ₂	361.0	299	-55	-25	6.4
15-HETrE	321.2	303	-70	-21	9.4	PGG ₂	367.2	113	-60	-30	15.0
8-HETrE	321.2	303	-70	-21	9.7	6k PGE ₁	367.2	143	-40	-25	2.8
2,3-dinor-PGE ₁	325.2	163	-60	-30	2.5	19oh PGE ₂	367.2	189	-40	-25	2.8
Tetranor-PGEM	327.0	291	-40	-25	13.8	δ17 6k-PGF _{1α}	367.2	207	-90	-35	3.7
DHA	327.2	299	-60	-30	13.4	20oh PGE ₂	367.2	349	-40	-25	2.8
Tetranor-PGFM	329.2	311	-40	-25	14.4	6,15 dk-,dh-PGF _{1α}	368.2	97	-60	-40	3.1
PGB ₃	331.2	235	-60	-30	3.2	6k-PGF _{1α}	369.2	245	-65	-35	3.3
Adrenic Acid	331.3	313	-60	-30	15.3	20oh PGF _{2α} /19oh PGF _{2α}	369.2	307	-50	-35	14.8

15d-PGD ₂	333.0	271	-30	-20	4.7	19oh PGE ₁	369.2	333	-60	-30	8.3
PGB ₂	333.2	235	-30	-20	5.4	dh-, 19 oh PGE ₁	371.2	353	-60	-30	2.8
bicyclo-PGE ₂	333.2	235	-30	-20	5.4	RvD ₁	375.0	141	-45	-20	4.6
dhk PGA ₂	333.2	265	-60	-30	13.6	dihomoPGE ₂	379.0	299	-65	-30	5.1
PGJ ₂	333.2	271	-30	-20	4.7	dihomo PGD ₂	379.0	299	-65	-30	5.4
5,15-diHETE	335.2	255	-50	-30	5.7	20-ethyl PGE ₂	379.2	343	-60	-30	15.3
LTB ₄	335.2	151	-70	-22	6.0	20-ethyl PGF _{2α}	381.3	337	-60	-30	15.2
5,6-diHETE	335.2	163	-60	-25	7.2	1α,1β-dihomo-PGF _{2α}	381.3	345	-60	-30	4.9
12-epi-LTB ₄	335.2	183	-40	-20	5.6	1α,1β-dihomo-PGE ₁	381.3	345	-60	-30	4.3
6t,12 epi-LTB ₄ /6t	335.2	195	-70	-22	5.6	PGD ₂	424.3	203	-60	-30	4.3
LTB ₄						dihydroxypropylamine					
Hepoxilin A ₃ /B ₃	335.2	195	-70	-22	7.3	PGF _{2α}	426.3	290	-60	-30	3.1
						dihydroxypropylamine					
5,6 DHET	337.0	145	-75	-25	7.7	14R-LXA ₄	438.0	333	-60	-25	3.6
8,9-diHETrE	337.2	111	-60	-30	12.7	11t-LTE ₄	438.0	333	-60	-25	5.3
11d-PGF _{1α} /11d-	339.3	321	-60	-30	3.2	LTD ₄ (W)	495.0	177	-60	-25	5.0
PGF _{1β}											
19,20 EpDPE	343.0	241	-70	-17	9.9	11t-LTD ₄	495.0	177	-60	-25	5.9
8-HDoHE	343.2	109	-70	-20	9.0	LTC ₄	624.3	306	-70	-28	4.9

Supplementary Table 3. The standard curve, linear ranges, sensitivity, and stability of ten lipid compounds detected by MRM.

Analytes	Calibration curve	R2	LOQ (ng/mL)	LOD (ng/mL)	Precision (n = 6) RSD%		Stability (n = 6) RSD%
					Intra-day	Inter-day	
PGF _{2α}	y=0.0057x+0.0126	0.9995	3.44	1.03	2.64	6.14	6.14
15-HETrE	y=0.0261x+0.0094	0.9999	2.87	0.86	0.96	2.62	4.59
9,10-diHOME	y=0.0273x+0.0386	0.9999	1.25	0.37	2.07	3.83	2.88
15-HETE	y=0.0148x+0.0361	0.9998	1.41	0.42	1.08	3.07	2.92
13-HODE	y=0.0199x+0.0809	0.9998	2.09	0.62	1.85	2.85	2.25
17,18 EpETE	y=0.0060x+0.0352	0.9999	5.51	1.65	2.65	2.68	5.91
10-HDoHE	y=0.0076x+0.0324	0.9999	3.76	1.12	2.20	2.93	3.00
9,10 EpOME	y=0.0132x+0.0628	0.9999	4.47	1.34	2.17	2.10	6.19
19,20 EpDPE	y=0.0038x+0.0205	0.9999	10.13	3.04	3.20	2.61	4.95
Arachidonic Acid	y=0.0001x+0.0171	0.9999	420.2	126.0	1.78	2.56	2.71

Supplementary Table 4. Accuracy and precision of LC-MS/MS method for quantifying eicosanoids in rat liver (n = 3)

Analytes	Spiked level(ng/mL)	Accuracy (%)	RSD (%)
15-HETrE	10	113.37	3.27
	25	108.94	1.85
	50	107.50	5.05
9,10-diHOME	10	105.74	5.76
	25	108.11	7.21
	50	107.09	9.25
10-HDoHE	30	100.72	5.08
	75	116.83	3.21
	150	117.72	2.22
9,10 EpOME	30	96.37	7.72
	75	94.42	6.00
	150	98.09	5.58
19,20 EpDPE	30	102.18	4.38
	75	102.61	6.79
	150	114.11	1.21
17,18 EpETE	30	91.44	3.05
	75	111.13	6.61
	150	108.06	5.16
PGF _{2α}	20	107.49	6.68
	50	105.28	18.48
	100	99.49	15.84
15-HETE	20	104.71	1.91
	50	104.07	12.37
	100	107.90	4.00

13-HODE	20	106.78	8.63
	50	87.72	3.91
	100	95.92	11.91
Arachidonic Acid	500	99.79	6.46
	1250	99.93	9.22
	2500	110.08	4.92

Accuracy (%)=[measured concentration – blank rat liver concentration)/spiked concentration]×
100%

RSD=STDEV(Cg-Cb)/AVERAGE(Cg-Cb)×100 (Cg: measured concentration; Cb: blank rat liver
concentration)

Supplementary Table 5. Effect of As₄S₄ exposure on target lipid profiling in rat liver
($\bar{x} \pm SD$, n=5-6, ng/g)

Fatty acid	Pathway	Name	Nor	As ₄ S ₄ (41 mg/kg)	As ₄ S ₄ (134 mg/kg)
AA	COX	PGD ₂ dihydroxy propylamine	9.61±5.92	7.39±2.59	8.66±4.22
AA	COX	dh PGF _{2α}	128.86±75.49	65.74±14.66	137.78±128.51
AA	COX	dhk PGD ₂	5.01±3.29	10.80±6.34	6.98±2.81
AA	COX	dhk PGF _{2α}	11.43±2.85	19.96±5.96*	21.15±7.16*
AA	COX	15k PGF _{2α}	19.38±5.49	38.08±11.25*	37.39±13.19*
AA	COX	dhk PGE ₂	49.81±8.94	90.26±26.78*	94.02±32.71*
AA	COX	PGJ ₂	16.98±6.18	33.43±11.66*	31.81±7.75**
AA	COX	15d-PGJ ₂	36.46±8.63	30.58±9.85	52.74±13.75
AA	COX	dhk-PGE ₁	12.58±1.48	13.21±2.41	12.75±4.57
AA	COX	19oh PGE ₁	26.94±14.10	15.83±6.22	20.34±10.08
AA	COX	PGK ₂	429.59±124.80	423.81±68.74	622.55±316.21
AA	COX	dhk PGF _{1α}	45.30±28.11	19.56±5.06	49.15±53.24
AA	COX	20oh PGF _{2α} /19oh PGF _{2α}	66.52±16.74	52.05±10.27	68.40±27.09
AA	COX	PGG ₂	56.20±4.48	62.18±14.75	59.55±16.55
AA	COX	PGF _{2α}	60.23±15.55	107.61±32.99*	111.66±40.01*
AA	COX	8-iso-PGF _{2α}	42.76±11.23	72.88±20.39*	75.03±24.41*
AA	COX	PGE ₂	507.42±159.13	1044.60±306.21**	995.83±328.30*
AA	COX	PGD ₂	181.87±41.38	394.60±120.62**	329.38±124.44*
AA	COX	15d-PGD ₂	14.63±5.54	26.94±7.39*	23.85±5.13*
AA	COX	20-ethyl PGF _{2α}	48.56±19.56	27.52±7.43*	45.32±27.64

AA	COX	tetranor-PGEM	109.30±45.07	69.10±15.11	109.24±65.46
AA	COX	tetranor-PGFM	961.82±601.03	422.01±123.84	768.03±584.78
AA	COX	PGD ₃	44.80±15.39	59.77±30.62	52.72±10.94
AA	COX	11-HEPE	22.22±9.56	34.91±18.03	28.29±11.74
AA	COX	11-HETE	306.87±89.95	593.43±196.06*	546.81±247.60
AA	COX	13-HDoHE	140.37±42.69	157.41±56.74	178.19±104.05
AA	LOX	15-HETE	74.37±23.53	113.95±33.25	112.38±57.07
AA	LOX	5-HETE	10.72±4.92	24.29±10.92*	19.07±8.41
AA	LOX	8-HETE	6.87±1.98	11.36±4.59	11.82±7.44
AA	LOX	12-HETE	8.31±3.72	15.25±6.88	12.77±8.27
LA	LOX	9-oxoODE	18.30±7.85	8.64±2.72*	22.45±16.86
LA	LOX	13-HpODE	211.40±135.81	105.63±36.50	153.92±69.22
LA	LOX	9-HODE	628.07±234.05	706.72±206.54	682.69±281.06
LA	LOX	9-HOTrE/13-HOTrE	37.31±16.91	34.55±13.80	37.07±13.12
LA	LOX	13-HODE	383.72±175.75	431.21±158.37	456.49±207.68
DHA	CYP	19,20 EpDPE	16.08±3.80	23.62±9.65	20.56±9.48
LA	CYP	12,13 EpOME	108.64±44.81	75.10±50.75	76.51±83.48
LA	CYP	9,10 EpOME	41.32±12.99	39.87±15.26	40.34±24.64
LA	CYP	9,10-diHOME	413.11±248.82	461.70±241.35	399.66±184.13
AA	CYP	8,9-diHETrE	46.77±14.33	45.79±9.38	59.91±12.46
AA	CYP	20-HETE	6.00±3.15	17.52±12.76	13.43±6.84
DHA	CYP	19,20-diHDPA	25.19±9.91	25.36±10.01	30.73±18.49
EPA	CYP	17,18 EpETE	12.07±4.04	22.13±9.18*	14.57±3.96
EPA		EPA	954.57±435.71	747.00±231.84	942.61±520.75
EPA	non-enz	9-HEPE	78.81±37.33	149.98±73.33	100.68±33.10
EPA	LOX	15-HEPE	19.40±8.92	30.37±12.79	23.25±8.04
EPA	LOX	5-HEPE	19.26±13.08	18.97±7.80	18.77±14.94
EPA	LOX	12-HEPE	14.08±7.15	30.21±18.38	15.01±7.71

EPA	CYP	18-HEPE	68.62±31.43	115.15±51.31	75.75±34.11
DHA		DHA	117.40±21.45	117.71±22.49	115.21±34.42
DHA	LOX	17-HDoHE	38.38±19.25	38.26±14.25	47.43±31.32
DHA	LOX	7-HDoHE	128.16±52.00	166.83±68.89	160.88±102.59
DHA	LOX	4-HDoHE	24.42±11.28	34.34±13.95	30.06±16.46
DHA	LOX	10-HDoHE	62.11±31.20	95.09±46.18	72.00±49.56
DHA	non-enz	8-HDoHE	11.63±7.68	19.05±8.39	15.11±11.05
DHA	non-enz	20-HDoHE	81.89±26.70	117.29±67.05	109.15±66.41
DγLA		DγLA	204.62±99.14	105.48±38.40*	220.12±188.23
DγLA	LOX	15-HETrE	30.69±13.05	54.88±17.79*	54.39±27.54
DγLA	LOX	8-HETrE	23.22±9.17	43.29±15.66*	40.32±22.08
AA		Arachidonic acid	926.40±257.56	884.80±141.34	1340.58±707.52
SDA		Stearidonic Acid	17.90±5.08	15.69±4.04	18.34±2.97
ADA		Adrenic Acid	166.16±101.48	63.22±15.48*	167.91±175.72

Values were expressed as mean ± SD (n=10), * P <0.05, ** P <0.01, compared with normal control.

