

Original article

Pretreatment with Tetrandrine Enhanced the Antioxidative and Immunomodulatory Activity of Mesenchymal Stem Cells

Shohreh Fadaghi¹, Merat Mahmoodi^{1,*}, Ali Derakhshani², Farnaz Sedghy¹, Mahdi ranjkesh¹, Ahmadreza Behzadi³

1. Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
2. Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran
3. School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.

***Corresponding authors:**

Merat Mahmoodi

Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.

Mailing Address:

Faculty of Medicine, Shahid Bahonar University, Pajooresh Sq, Kerman, Iran

Postal Code: 76169-133

Phone: +98 3433257660

Email: merat.mahmoodi@yahoo.com

Table 1s: effect of tetrandrine on the proliferation of MSC cells

Tetrandrine Con. (μM)	Non-Treated	50(μM)	10(μM)	5 (μM)	DMSO-treated
OD₅₇₀	87.59558	17.16228	96.94138	107.7315	8.581138
	100.8496	15.548	101.6992	99.57519	7.22175
	109.8556	24.55395	84.96177	117.5871	8.496177
	101.7842	25.23364	114.5285	94.2226	8.241291
	100	16.90739	105.6075	103.9082	8.135089

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (50 μM)	80.14	65.90 to 94.37	Yes	****	<0.0001
Non-treated vs. Tetrandrine (10 μM)	-0.7307	-14.97 to 13.50	No	ns	0.9999
Non-treated vs. Tetrandrine (5 μM)	-4.588	-18.82 to 9.648	No	ns	0.8677
Non-treated vs. DMSO-treated	91.88	77.65 to 106.1	Yes	****	<0.0001
Tetrandrine (50 μM) vs. Tetrandrine (10 μM)	-80.87	-95.10 to -66.63	Yes	****	<0.0001
Tetrandrine (50 μM) vs. Tetrandrine (5 μM)	-84.72	-98.96 to -70.49	Yes	****	<0.0001
Tetrandrine (50 μM) vs. DMSO-treated	11.75	-2.490 to 25.98	No	ns	0.1380
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	-3.857	-18.09 to 10.38	No	ns	0.9242
Tetrandrine (10 μM) vs. DMSO-treated	92.61	78.38 to 106.8	Yes	****	<0.0001
Tetrandrine (5 μM) vs. DMSO-treated	96.47	82.23 to 110.7	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (50 μM)	100.0	19.88	80.14	4.757	5	5	23.82	20
Non-treated vs. Tetrandrine (10 μM)	100.0	100.7	-0.7307	4.757	5	5	0.2172	20
Non-treated vs. Tetrandrine (5 μM)	100.0	104.6	-4.588	4.757	5	5	1.364	20
Non-treated vs. DMSO-treated	100.0	8.135	91.88	4.757	5	5	27.31	20
Tetrandrine (50 μM) vs. Tetrandrine (10 μM)	19.88	100.7	-80.87	4.757	5	5	24.04	20
Tetrandrine (50 μM) vs. Tetrandrine (5 μM)	19.88	104.6	-84.72	4.757	5	5	25.19	20
Tetrandrine (50 μM) vs. DMSO-treated	19.88	8.135	11.75	4.757	5	5	3.492	20
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	100.7	104.6	-3.857	4.757	5	5	1.147	20
Tetrandrine (10 μM) vs. DMSO-treated	100.7	8.135	92.61	4.757	5	5	27.53	20
Tetrandrine (5 μM) vs. DMSO-treated	104.6	8.135	96.47	4.757	5	5	28.68	20

Table 2s: effect of tetrandrine on the viability of MSC cells

Tetrandrine Con. (μM)	Non-treated	10 μM	5 μM
Viability %	98	96.9	99.1
	98.2	98.3	98.3
	99	97.2	97.6
	100	98.8	99.2
	98	99.2	98.9

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	0.5600	-0.8823 to 2.002	No	ns	0.5695
Non-treated vs. Tetrandrine (5 μM)	0.02000	-1.422 to 1.462	No	ns	0.9992
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	-0.5400	-1.982 to 0.9023	No	ns	0.5914

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	98.64	98.08	0.5600	0.5406	5	5	1.465	12
Non-treated vs. Tetrandrine (5 μM)	98.64	98.62	0.02000	0.5406	5	5	0.05232	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	98.08	98.62	-0.5400	0.5406	5	5	1.413	12

Table 3s: effect of tetrandrine on the cell cycle of MSC cells

Cell cycle	G1			S			G2/M		
Non-treated	72.59	72.33	72.46	8.88	10.06	9.47	6.57	6.60	6.58
Tetrandrine(10 μ M)	73.57	69.30	71.43	10.11	11.36	10.73	4.77	7.08	5.92

Table 4s: effect of tetrandrine on the immunophenotype of MSC cells

Markers	Non-treated			Tetrandrine(10 μ M)		
CD29	99.15	99.2	99.1	99.4	99.100	99.300
CD90	98.5	99.2	99.1	98.65	98.200	99.100
CD45	0.461	0.302	0.405	0.581	0.347	0.364
HLA-DR	0.595	0.607	0.602	0.706	0.575	0.410

Table 5s: effect of tetrandrine on the anti-oxidant activities of MSC cells

Tetrandrine Con. (μ M)	Non-treated	Tetrandrine(10 μ M)	Tetrandrine(5 μ M)
SOD3 level (U/mL) OD ₅₆₀	2.76	2.760	3.680
	2.606	2.453	3.985
	2.300	3.577	3.373
	1.993	3.015	3.679
	2.732	3.001	3.243

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μ M)	-0.4830	-1.069 to 0.1034	No	ns	0.1120
Non-treated vs. Tetrandrine (5 μ M)	-1.114	-1.700 to -0.5274	Yes	***	0.0007
Tetrandrine (10 μ M) vs. Tetrandrine (5 μ M)	-0.6308	-1.217 to -0.04442	Yes	*	0.0350

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	2.478	2.961	-0.4830	0.2198	5	5	3.108	12
Non-treated vs. Tetrandrine (5 μM)	2.478	3.592	-1.114	0.2198	5	5	7.166	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	2.961	3.592	-0.6308	0.2198	5	5	4.059	12

Tetrandrine Con. (μM)	Non-treated	Tetrandrine(10 μM)	Tetrandrine(5 μM)
MDA level (nmol/ml) OD ₅₄₀	2.7640	2.992	2.63300
	2.5080	2.433	2.41400
	2.3260	2.978	2.88500
	2.7920	2.913	2.57700
	2.5975	2.829	2.62725

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	-0.2315	-0.5670 to 0.1040	No	ns	0.1983
Non-treated vs. Tetrandrine (5 μM)	-0.02975	-0.3652 to 0.3057	No	ns	0.9697
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	0.2018	-0.1337 to 0.5372	No	ns	0.2814

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	2.598	2.829	-0.2315	0.1257	5	5	2.604	12
Non-treated vs. Tetrandrine (5 μM)	2.598	2.627	-0.02975	0.1257	5	5	0.3346	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	2.829	2.627	0.2018	0.1257	5	5	2.269	12

Table 6s: effect of tetrandrine on the IDO activity of MSC cells

Tetrandrine Con. (μM)	Non-treated	Tetrandrine(10 μM)	Tetrandrine(5 μM)
IDO Activity OD ₄₉₂	0.237	0.245	0.266
	0.231	0.252	0.262
	0.234	0.247	0.262
	0.236	0.244	0.263
	0.240	0.240	0.267

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	-0.01000	-0.01585 to -0.004147	Yes	**	0.0018
Non-treated vs. Tetrandrine (5 μM)	-0.02840	-0.03425 to -0.02255	Yes	****	<0.0001
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	-0.01840	-0.02425 to -0.01255	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	0.2356	0.2456	-0.01000	0.002194	5	5	6.446	12
Non-treated vs. Tetrandrine (5 μM)	0.2356	0.2640	-0.02840	0.002194	5	5	18.31	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	0.2456	0.2640	-0.01840	0.002194	5	5	11.86	12

Table 7s: effect of tetrandrine on the Cytokines secretion of MSC cells

Tetrandrine Con. (μM)	Non-treated	Tetrandrine(10 μM)	Tetrandrine(5 μM)
IFN- γ levels(pg/ml)	1.5	1.7	1.2
	1.5	1.5	1.3
	2.2	1.8	1.0
	1.8	1.6	1.5
	1.7	1.6	1.6

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	0.1000	-0.2810 to 0.4810	No	ns	0.7679
Non-treated vs. Tetrandrine (5 μM)	0.4200	0.03895 to 0.8010	Yes	*	0.0308
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	0.3200	-0.06105 to 0.7010	No	ns	0.1043

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	1.740	1.640	0.1000	0.1428	5	5	0.9901	12
Non-treated vs. Tetrandrine (5 μM)	1.740	1.320	0.4200	0.1428	5	5	4.159	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	1.640	1.320	0.3200	0.1428	5	5	3.168	12

Tetrandrine Con. (μM)	Non-treated	Tetrandrine(10 μM)	Tetrandrine(5 μM)
TGF-B level(pg/ml)	5.471	6.000	10.090
	4.516	7.079	8.398
	7.577	6.391	7.724
	7.090	7.157	5.358
	5.092	6.656	4.310

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	-0.7074	-3.362 to 1.947	No	ns	0.7618
Non-treated vs. Tetrandrine (5 μM)	-1.227	-3.881 to 1.428	No	ns	0.4574
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	-0.5194	-3.174 to 2.135	No	ns	0.8622

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	5.949	6.657	-0.7074	0.9950	5	5	1.005	12
Non-treated vs. Tetrandrine (5 μM)	5.949	7.176	-1.227	0.9950	5	5	1.744	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	6.657	7.176	-0.5194	0.9950	5	5	0.7382	12

Table 8s: effect of tetrandrine pre-treated MSC on the proliferation of PBMC cells

Tetrandrine Con. (μM)	Non-treated	Tetrandrine(10 μM)	Tetrandrine(5 μM)
OD₅₇₀	2.38	1.103864	1.615227
	2.33	1.643864	1.065227
	2.00	1.243864	0.725227
	1.99	1.623864	1.205227
	2.17	1.623864	1.315227

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	0.7261	0.2852 to 1.167	Yes	**	0.0023
Non-treated vs. Tetrandrine (5 μM)	0.9888	0.5479 to 1.430	Yes	***	0.0002
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	0.2626	-0.1783 to 0.7036	No	ns	0.2875

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	2.174	1.448	0.7261	0.1653	5	5	6.213	12
Non-treated vs. Tetrandrine (5 μM)	2.174	1.185	0.9888	0.1653	5	5	8.461	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	1.448	1.185	0.2626	0.1653	5	5	2.247	12

Table 9s: effect of tetrandrine pre-treated MSC on the cell cycle of PBMC cells

Cell cycle	G1			S			G2/M		
Non-treated	78.17	84.91	83.73	12.51	11.78	11.03	1.00	0.60	0.78
Tetrandrine(5 μM)	82.46	83.11	83.28	5.91	6.99	6.35	0.20	0.47	0.33

Table 10s: effect of tetrandrine pre-treated MSC on the cytokines secretion of PBMC cells

Tetrandrine Con. (μM)	Non-treated	Tetrandrine(10 μM)	Tetrandrine(5μM)
IFN-γ level(pg/ml)	190.4	48.6	40.0
	227.0	60.1	62.0
	184.7	197.2	130.5
	231.2	96.2	184.6
	173.5	141.5	104.3

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	92.64	7.132 to 178.1	Yes	*	0.0337
Non-treated vs. Tetrandrine (5 μM)	97.08	11.57 to 182.6	Yes	*	0.0263
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	4.440	-81.07 to 89.95	No	ns	0.9895

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μM)	201.4	108.7	92.64	32.05	5	5	4.088	12
Non-treated vs. Tetrandrine (5 μM)	201.4	104.3	97.08	32.05	5	5	4.284	12
Tetrandrine (10 μM) vs. Tetrandrine (5 μM)	108.7	104.3	4.440	32.05	5	5	0.1959	12

Tetrandrine Con. (μM)	Non-treated	Tetrandrine(10 μM)	Tetrandrine(5μM)
TGF-B level(pg/ml)	4.512	8.920	7.987
	6.326	9.527	7.744
	5.784	6.391	11.210
	4.575	7.846	9.110
	4.819	7.825	7.139

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Non-treated vs. Tetrandrine (10 μM)	-2.899	-5.005 to -0.7925	Yes	**	0.0083
Non-treated vs. Tetrandrine (5μM)	-3.435	-5.541 to -1.329	Yes	**	0.0025

Tetrandrine (10 μ M) vs. Tetrandrine (5 μ M)	-0.5362	-2.642 to 1.570	No	ns	0.7797
--	---------	-----------------	----	----	--------

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Non-treated vs. Tetrandrine (10 μ M)	5.203	8.102	-2.899	0.7894	5	5	5.193	12
Non-treated vs. Tetrandrine (5 μ M)	5.203	8.638	-3.435	0.7894	5	5	6.153	12
Tetrandrine (10 μ M) vs. Tetrandrine (5 μ M)	8.102	8.638	-0.5362	0.7894	5	5	0.9606	12

Table 11s: effect of tetrandrine pre-treated MSC on the Treg cell percentage in PBMC cells

Tetrandrine Con. (μ M)	Non-treated			Tetrandrine (5 μ M)		
Treg cells (%)	1.96	1.71	1.83	2.64	1.02	1.35

Unpaired t test	
P value	0.7601
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.3270, df=4