

**Table 9** Proteins identified by mass spectrometry

Spot No.	Accession No.	Protein Identification	MW(KDa)/pI	Score	Post-translational modification
1		Beta-lactoglobulin	18.3 / 4.8	64	
2	P16045	Galectin-1	14.7 / 5.3	146	
3	P70296	Phosphatidylethanolamine-binding protein	20.7 / 5.2	76	
4	O70251	Elongation factor 1-beta	24.5 / 4.5	42	
7		Beta-actin	39.1 / 5.8	75	
8	P02554	Tubulin beta chain	49.8 / 4.8	67	
11		Pyruvate dehydrogenase (lipoamide) beta	38.9 / 6.4	256	
12	P62739	Alpha-actin 2	42.0 / 5.2	48	
13	P56480	ATP synthase beta chain	56.3 / 5.2	588	
14		Calreticulin	48.0 / 4.3	130	
15	P63038	60 kDa heat shock protein	60.9 / 5.9	759	Oxidation (M55, M256, M477)
16		Tumor rejection antigen gp96	92.4 / 4.7	307	
17		Beta-actin	39.2 / 5.8	266	Oxidation (M299)
18	P63038	60 kDa heat shock protein	60.9 / 5.9	585	
19	P38647	Stress-70 protein	73.5 / 5.9	580	Deamidation (N268)
20	P27773	Protein disulfide-isomerase A3	56.6 / 6.0	426	
21	P08228	Superoxide dismutase [Cu-Zn]	15.8 / 6.0	169	
22	P70349	Histidine triad nucleotide-binding protein 1	13.6 / 6.4	70	
24	Q64433	10 kDa heat shock protein	10.8 / 8.2	70	
27	P00348	Short chain 3-hydroxyacyl-CoA dehydrogenase	34.1 / 9.0	63	
28	Q60930	Voltage-dependent anion-selective channel protein 2	31.7 / 7.4	245	
30		Aconitase 2	85.4 / 8.1	990	Oxidation (m34, M393) Deamidation (N626)
31	P62962	Profilin I	14.8 / 8.5	150	Deamidation (N61)
32	Q60932	Voltage-dependent anion-selective channel protein 1	32.3 / 8.6	373	Deamidation (N252)
33	P05202	Aspartate aminotransferase (Transaminase A)	47.4 / 9.1	261	Deamidation (N322)
34	Q8CAQ8	Mitochondrial inner membrane protein (Mitofilin)	83.8 / 6.2	377	
35	Q9QZ23	HIRA-interacting protein 5	22.1 / 4.2	91	
38	P70580	Membrane associated progesterone receptor component 1	21.5 / 4.5	57	
39	P63028	Translationally controlled tumor protein	19.5 / 4.8	120	
40	P70296	Phosphatidylethanolamine-binding protein	20.7 / 5.2	138	
41	O35658	Complement component 1, Q subcomponent binding protein	31.0 / 4.8	79	

42	Q61937	Nucleophosmin	32.5 / 4.6	313	
44	P09103	Protein disulfide-isomerase precursor	57.1 / 4.8	780	Oxidation (M326, M358, M427)
45	Q9R0P9	Ubiquitin carboxyl-terminal hydrolase isozyme L1	24.8 / 5.1	119	
46	P11177	Pyruvate dehydrogenase E1 component beta subunit	39.2 / 6.2	74	
47		G protein beta 1 subunit	37.4 / 5.5	308	
49	Q63081	Protein disulfide-isomerase A6 precursor	47.2 / 5.0	144	
50	P52597	Heterogeneous nuclear ribonucleoprotein F	45.5 / 5.4	155	
51		Beta-actin	39.1 / 5.8	99	
52	Q9CZ13	Ubiquinol-cytochrome-c reductase complex core protein I	52.7 / 5.8	414	Deamidation (N86) Oxidation (M229, M440, M475)
53	P63038	60 kDa heat shock protein	60.9 / 5.9	770	Oxidation (M55, M40, M146, M217, M230, M316, M356, M513, M506) Deamidation (N136, Q496)
54	P63038	60 kDa heat shock protein	60.9 / 6.0	156	
55		Hsc70-ps1	70.9 / 5.4	516	Oxidation (m61, M549) Deamidation (N57, N355)
57	P50213	Isocitrate dehydrogenase [NAD] subunit alpha	39.6 / 6.5	181	
58	P18242	Cathepsin D precursor	44.9 / 6.7	88	
59		Hypothetical protein 4732456N10	58.2 / 8.5	75	Deamidation (N307)
60	P15331	Peripherin	54.2 / 5.4	490	Oxidation (M863, M391)
61	P31943	Heterogeneous nuclear ribonucleoprotein H	49.1 / 5.9	74	
62	P38647	Stress-70 protein	73.5 / 5.9	662	Deamidation (N149, N188) Oxidation (M172)
63	P27773	Protein disulfide-isomerase A3	56.6 / 6.0	505	Phospho (Y67, H126, S149, H167, Y478) Deamidation (N212, N473) Oxidation (M337, M433)
65	P68002	Voltage-dependent anion-selective channel protein 2	31.6 / 7.5	86	
66	Q15365	Poly(rC)-binding protein 1	37.5 / 6.7	114	
67	P18242	Cathepsin D	44.9 / 6.7	119	
71	P14851	Peptidyl-prolyl cis-trans isomerase A	17.8 / 8.5	37	
72	P02662	Alpha-S1 casein precursor	24.5 / 5.0	50	
74	Q9D0K2	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1	56 / 8.3	178	Oxidation (M111) Deamidation (N482)
75	Q9DCW4	Electron transfer flavoprotein beta-subunit	27.3 / 8.6	162	
77	Q8QZT1	Acetyl-CoA acetyltransferase	44.8 / 8.7	179	
78	P04075	Fructose-bisphosphate aldolase A	39.3 / 8.4	63	
79	Q04467	Isocitrate dehydrogenase	50.8 / 9.0	35	

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80	P97807	Fumarate hydratase	54.3 / 9.1	190	
81		Heterogeneous nuclear ribonucleoprotein A2/B1 isoform 1	36.0 / 8.7	130	Oxidation (M41)
82	P05202	Aspartate aminotransferase	47.4 / 9.1	162	Oxidation (M281)
83	P05202	Aspartate aminotransferase	47.4 / 9.1	180	Deamidation (N322)
84	P19483	ATP synthase alpha chain heart isoform	59.7 / 9.2	170	

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**Table 10** Important proteins identified from mass spectrometry and the results of QPCR

Spot No.	Protein Identification	Functional Classification	QPCR (NMDA+aloe-emodin metabolites – NMDA)
2	Galectin-1	This protein binds beta-galactoside. Its physiological function is not yet known. It may act as an autocrine negative growth factor that regulates cell proliferation	— <sup>§</sup>
4	Elongation factor 1-beta	EF-1-beta and EF-1-delta stimulate the exchange of GDP bound to EF-1-alpha to GTP. Phosphorylation affects the GDP/GTP exchange rate	—
15	60 kDa heat shock protein	Implicated in mitochondrial protein import and macromolecular assembly. It interacts with p21Ras	—
19	Stress-70 protein	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.	—
21	Superoxide dismutase [Cu-Zn]	Destroys radicals which are normally produced within the cells and which are toxic to biological systems. $2 \text{ superoxide} + 2 \text{ H}^+ = \text{O}_2 + \text{H}_2\text{O}_2$	+ *
24	10 kDa heat shock protein	Eukaryotic CPN10 homolog which is essential for mitochondrial protein biogenesis, together with CPN60. Binds to CPN60 in the presence of Mg-ATP and suppresses the ATPase activity	+
32	Voltage-dependent anion-selective channel protein 1	Forms a channel through the mitochondrial outer membrane and also the plasma membrane	—
34	Mitochondrial inner membrane protein (Mitofilin)		—
44	Protein disulfide-isomerase A6 precursor	Catalyzes the rearrangement of –S-S-bonds in proteins	+

§ “–”: decrease      \* “+”: increase

**Table 11** Primers and condition for Q-PCR

GAPDH	5'-AGCTTGTCATCAACGGGAAG-3' 5'-TTTGATGTTAGTGGGGTCTCG-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
Galectin-1	F: 5'-CTTCGCTTCATCATCATGGCCT-3' R: 5'-AATGGTGTGGCATCTCCGT-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
EF 1-β	F: 5'-TGATAAGGTTGGAACAGATTTGC-3' R: 5'-GACTGTACGTAGTCCTCAAAGCA-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
Hsp60	F: 5'-AGGAAAAGCTGAACGAGCGA-3' R: 5'-ATGAATCCAAGGCTGGGATG-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
Stress-70	F: 5'-GGTAAGATTTTCACCCCTGAAG-3' R: 5'-TGTA AACCTTAAATGGGTATCTGC-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
Cu-Zn SOD	F: 5'-CATTCCCTATGTGGTCTGAGTCT-3' R: 5'-CAGTTTAATGGTTTGGTTTCTACAGT-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
Hsp10	F: 5'-GAGGGGCTTGTTCTTTCACC-3' R: 5'-AAGGGGAGCACTTTCTAGGC-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 60°C for 60 sec
VDAC-1	F: 5'-GGATTTGGTTTTGGGTTGGT-3' R: 5'-TTCCACACCACTGCATGACT-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
Mitofilin	F: 5'-TAGCGCAACAGAAAGCCACA-3' R: 5'-TCCGCATTTCACTCTCCATG-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec
PDI A6 precursor	F: 5'-AGGTGAAATTGGCAGCCGTA-3' R: 5'-CCCTTGACACTATGTCAGATCTCGT-3' 95°C for 10 min 40 cycles; 95°C for 15 sec, 59°C for 60 sec

Abbreviation: elongation factor 1-beta (EF 1-β), 60 kDa heat shock protein (Hsp 60) , superoxide dismutase [Cu-Zn] (Cu-Zn SOD), 10 kDa heat shock protein (Hsp 10), voltage-dependent anion-selective channel protein 1 (VDAC-1), mitochondrial inner membrane protein (Mitofilin) and protein disulfide-isomerase (PDI) A6 precursor.

**Table 12** The results of QPCR

treatment	NMDA (a) <sup>ψ</sup>	NMDA+ aloe-emodin s/g (b) <sup>Ω</sup>	a-b <sup>θ</sup>	p value
2- ΔΔD				
galectin-1	1.21 ± 0.07	0.93 ± 0.12	— <sup>ξ</sup>	0.025*
EF 1-β	0.89 ± 0.11	0.78 ± 0.09	—	0.25
Hsp 60	0.80 ± 0.03	0.75 ± 0.08	—	0.37
stress-70 protein	0.92 ± 0.10	0.85 ± 0.08	—	0.42
Cu-Zn SOD	0.71 ± 0.08	1.15 ± 0.05	+ <sup>π</sup>	0.01*
Hsp 10	0.64 ± 0.03	0.61 ± 0.06	+	0.48
VDAC-1	0.83 ± 0.04	0.79 ± 0.09	—	0.52
Mitofilin	1.35 ± 0.05	0.93 ± 0.09	—	0.02*
PDI A6 precursor	0.72 ± 0.03	0.68 ± 0.08	+	0.46

<sup>ψ</sup>a: The results of NMDA-treated N18 RGCs

<sup>Ω</sup>b: The results of NMDA and aloe-emodin s/g -treated N18 RGCs

<sup>θ</sup>a-b: The difference between the two

<sup>π</sup> “+”: a>b

<sup>ξ</sup> “—”: b>a

\*  $p < 0.05$