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Document Registration Number	2024-5
Full Product Name	SCORPION VENOM
Company Name	MFY ORTAKLAR GRUP LDT ŞTİ
Product Representative	Dilek DAĞLI Taylan YAVUZ
Sample Arrival Date	12.02.2024
Reason for Sample Arrival	Analysis of scorpion venom content and biological activity
Sample Information	Sample Description: Scorpion venom, 1 ml of white liquid
Sample Purity	%100
Scorpion Species	*Androctonus crassicauda* 
Analyses Conducted	1. Proteomic Analysis of Scorpion Venom 2. Metabolite Profiling of Scorpion Venom 3. Cytotoxicity Analysis of Scorpion Venom on Normal Cells
Evaluation	Attached are the analyses of the content and biological activity of the scorpion venom.

Date	Analyst	Responsible/Authorized
April 24, 2024	Özgür YÜKSEKDAĞ	Assoc. Prof. Dr. İsmail KOYUNCU

Scorpion Venom Analysis Report

INVESTIGATION OF THE PROTEOMIC STRUCTURE OF SCORPION VENOM

Visualization of Scorpion Venom using SDS-PAGE

Visualization of Scorpion Venom using SDS-PAGE: The protein concentrations of the scorpion venom samples were measured using the modified Bradford assay method (BioRad, USA). The accuracy of the measurements and the quality of the proteins were verified through SDS-PAGE analysis. The electrophoretic separation of the scorpion venom was carried out using a vertical electrophoresis system composed of 4-12% acrylamide under reducing conditions.

Protein Digestion and Peptide Acquisition Using the FASP Method

For the FASP digestion, 300 ng of protein was added to 300 µL of 8M urea solution and transferred onto a 30 kDa cutoff membrane filter placed within special microcentrifuge tubes. The mixture was centrifuged at 14,000 x g for 15 minutes at room temperature, and the filtrate below the filter was discarded. Then, 200 µL of 8M urea solution was added to the filter and centrifuged again. Iodoacetamide (IAA) solution was added to the filter and incubated in the dark for 20 minutes followed by centrifugation. After incubation, the filter was washed three times with 100 µL of 8M urea solution followed by centrifugation each time. For the washing step, the filter was washed three times with 100 µL of 50 mM Ammonium Bicarbonate (AmBic) solution and centrifuged again. The filter was then transferred to a clean microcentrifuge tube, to which trypsin enzyme and 50 mM AmBic solution were added, and digested overnight at 37°C. After digestion, the samples were eluted twice with 40 µL of 50 mM AmBic solution and once with 50 µL of 0.5 M NaCl solution, and collected into clean microcentrifuge tubes. Peptide samples were concentrated into a pellet using a SpeedVac vacuum centrifuge (Eppendorf, USA). The resulting peptides were resuspended in 20 µL of 0.1% Formic Acid (FA) solution for nLC-MS/MS analysis.

Nano-Liquid Chromatography Tandem Mass Spectrometry (nLC-MS/MS) Analysis

Peptides were analyzed using the nLC-MS/MS system with a Q-Exactive mass spectrometer (Thermo Scientific, USA) connected to an Ultimate 3000 RSLC nano system (Dionex, Thermo Scientific, CA, USA). The entire system was controlled through Xcalibur 4.0 software (Thermo Fisher Scientific, CA, USA). High-performance liquid chromatography (HPLC) separation was performed using mobile phases A (0.1% Formic Acid) and B (80% Acetonitrile + 0.1% Formic Acid). The digested peptides were concentrated and desalted on a trap column before being transferred to an Acclaim PepMap RSLC C18 analytical column (75 µm x 15 cm x 2 µm, 100 Å pore size, Thermo Scientific, CA, USA) for chromatographic separation. Full scan MS1 spectra were

acquired with the following parameters: resolution 70,000, scan range 400-2000 m/z, automatic gain control (AGC) target 3×10^6, maximum injection time 60 ms, spray voltage 2.3 kV. MS/MS analysis was conducted through data-dependent acquisition of the top ten precursor ions. MS2 analysis resulting from collision-induced dissociation (higher-energy collisional dissociation - HCD) used the following settings: resolution 17,500, AGC 1×10^6; maximum injection time 100 ms, isolation window 2.0 m/z, normalized collision energy (NCE) 27. The device was calibrated before each analysis using a standard positive calibrator (LTQ Velos ESI Positive Ion Calibration Solution 88323, Pierce, USA).

nLC-MS/MS Data Analysis

The analysis of raw data and protein identification were performed using Proteome Discoverer 2.2 software (Thermo Scientific, USA). The following parameters were applied during analysis: peptide mass tolerance of 10 ppm, MS/MS mass tolerance of 0.2 Da, mass accuracy of 2 ppm, low tolerance 1, minimum peptide length of 6 amino acids, fixed modifications included cysteine carbamidomethylation, variable modifications included methionine oxidation and asparagine deamination. A minimum of one unique peptide per protein was required for identification, and the resulting data were searched against the Uniprot database.

INVESTIGATION OF THE METABOLITE PROFILE OF SCORPION VENOM

Analysis of the Free Amino Acid Profile of Scorpion Venom by LC-MS/MS Method

The free amino acid profile of the venom samples was analyzed using LC-MS/MS (Shimadzu-8045) on samples prepared according to the protocol of a commercial kit (Bome Trivitron, Trimaris-BR130030, Turkey). This method uses a derivatization approach for the analysis of free amino acids. Initially, 100 µL of venom sample is mixed with an internal standard mixture containing 20 amino acids labeled with C13 and N15, prepared in 0.1 M HCl. In the second stage, organic buffer components with basic properties, prepared in propanol, are added to balance the pH and enhance the efficiency of the derivatization reaction. This stage also precipitates the proteins in the sample. Subsequently, a mixture containing 5% alkyl chloroformate in chloroform/isooctane is added as the active component and the mixture is allowed to stand at room temperature for 3 minutes. The derivatized amino acids are transferred to the upper phase containing organic solvents by centrifugation. A 1 µL injection from this phase is made into the LC-MS/MS system. The extraction and derivatization process increases the molecular weight and volatility of the amino acids, enhancing their signal in the MS device. Chromatographic separation is performed on a Trimaris Amino Acid LC-MS/MS column (250mm x 2mm, 3µM) containing a C18 reverse phase filler. Mobile phase A consists of Water: MeOH:1M Ammonium formate (85:14:1), and mobile phase B

consists of MeOH. Amino acid molecules are analyzed in MRM mode using ESI (+) ionization method.

Investigation Of The Carnitine-Acyl Carnitine Profile Of Scorpion Venom By Lc-Ms/Ms

The carnitine and acyl carnitine profile of the venom samples was analyzed by modifying the neonatal screening method developed by La Marca et al. (1). A 100 µL sample of scorpion venom was applied to Guthrie cards and left to dry at room temperature before being punched into 3.2 mm discs and placed into 96-well plates. Subsequently, each well received 300 µL of an extraction solution made of methanol and a 3 mmol/L aqueous hydrazine solution, and the samples were incubated at 37°C for 25 minutes to ensure butylation and extraction. Internal standards, consisting of stable heavy isotope analogs of carnitine and acyl carnitine [Labeled Carnitine Standards Set B (Cambridge Isotope Laboratories)], were added to the extracted solution. A 1 µL injection from this phase was made into the LC-MS/MS system. All collected data were reprocessed using Shimadzu Neonatal Software, which automatically calculates the concentration of each component.

INVESTIGATION OF THE CYTOTOXIC EFFECT OF SCORPION VENOM ON NORMAL HEALTHY CELLS (IC50)

Cells Used in the Study

Cells obtained from ATCC, including normal lung (BEAS-2B), kidney epithelial (HEK-293), endothelial (HUVEC), normal breast cell (CRL-4010), and normal prostate (PNT1-A) were used. The cell lines were incubated in DMEM-F12 and RPMI-1640 media, supplemented with 10% FBS and 1% glutamine, in a 5% CO2 atmosphere at 37°C. Following ATCC's recommendations, cells were detached using a mixture of 0.25% trypsin and 0.03% EDTA. The cells' growth and nutrition were supported using DMEM-F12 medium containing 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin. Surface-dependent cells were routinely subcultured every two days using a sterile glass Pasteur pipette until the surface was completely covered, followed by the addition of fresh sterile medium. Cells were allowed to proliferate in 75 cm3 cell culture flasks in a 37°C incubator with 5% CO2.

Determination of the Cytotoxic Effect of Venom Samples Using the MTT Method

The cytotoxic effect of scorpion venom on different healthy cells was examined using the MTT cell proliferation reagent. Cells were thawed, seeded in 25 cm3 flasks, and once 80-90% confluent, were detached via trypsinization and seeded into 96-well sterile plates at a density of 1x104 cells/well. After 24 hours, the medium was removed, and scorpion venom was applied at doses of 0, 2.5, 5, 10, 25, 50, 100, and 200 µg/ml to determine the cytotoxic dose (IC50). Each well received 200 µL, and then was incubated for 24 hours at 37°C in a 5% CO2 atmosphere. Post-incubation, the medium was removed, 90 µL of MTT solution was added to 200 µL of serum-free medium per well, and incubated for approximately 4 hours. After incubation, the medium was removed, formazan crystals were dissolved in 100 µL of DMSO, and absorbance values were measured and recorded at 470 and 690 nm wavelengths using a spectrophotometer.

References

1- La Marca G, Malvagia S, Pasquini E, Innocenti M, Fernandez MR, Donati MA, Zammarchi E. The inclusion of succinylacetone as marker for tyrosinemia type i in expanded newborn screening programs. Rapid Commun Mass Spectrom 2008; 22: 812-818).

RESULTS

PROTEOMIC STRUCTURE RESULTS OF SCORPION VENOM

SDS-PAGE Analyses of Scorpion Venom

The electrophoretic separation of scorpion venom was carried out using a vertical electrophoresis system composed of 4-12% acrylamide under reducing conditions. The SDS-PAGE image is provided in Figure-1. Although the SDS-PAGE analysis identified a high level of proteins in the 45-25 kDa range, it was observed that the majority of proteins consisted of peptides under 10 kDa. This image indicates that the scorpion venom sample is rich in peptides.

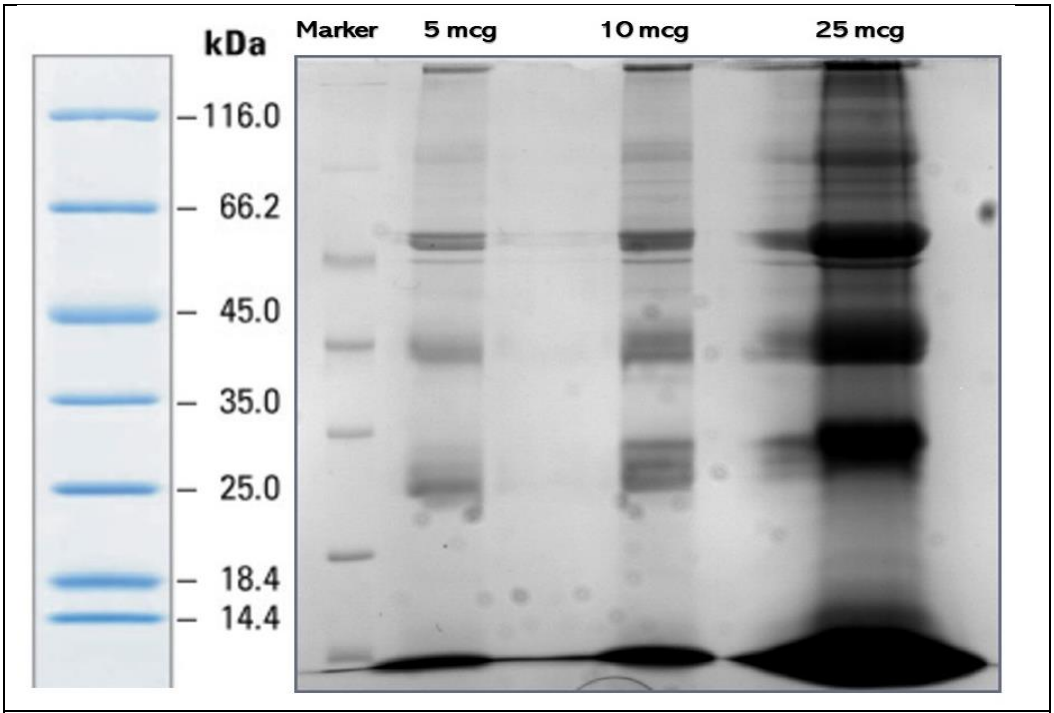

Fig 1. SDS-PAGE image of scorpion venom sample

Proteomic Profile Analysis of Scorpion Venom

The protein profile present in the scorpion venom sample was identified using the nLC-MS/MS analytical method, with results presented in Table 1. The analysis confirmed the presence of proteins, definitively classified as "Master proteins," within the venom sample. In total, 81 master proteins were identified. Notably, the presence of peptides such as Toxin Acra II, Sodium channel alpha-toxin Acra4, and Toxin b subunit alpha indicates a high purity level of the venom sample.

No	Protein type	*Accession*	*Description*	*Coverage [%]*	*# Peptides*	*# PSMs*	*# Unique Peptides*	*# Protein Groups*	*# AAs*	*MW [kDa]*	*calc. pI*	*Score Sequest HT: Sequest HT*	*# Peptides (by Search Engine): Sequest HT*
1.	Master Protein	P80476	Hemocyanin AA6 chain OS=Androctonus australis OX=6858 PE=1 SV=1	50	29	126	9	1	626	71,7	5,82	110,12	29
2.	Master Protein	A0A2I9LP22	Hemocyanin AA6 chain OS=Centruroides hentzi OX=88313 PE=3 SV=1	27	20	95	1	1	627	72	5,77	76,27	20
3.	Master Protein	A0A7T9L322	Hyaluronidase OS=Androctonus crassicauda OX=122909 GN=Hyal PE=2 SV=1	35	15	71	11	1	410	47,5	8,37	62,9	15
4.	Master Protein	P0C297	Toxin Acra II-3 OS=Androctonus crassicauda OX=122909 PE=2 SV=1	60	3	61	2	1	72	8,1	7,58	50,99	3
5.	Master Protein	P01486	Alpha-toxin Bot11 OS=Buthus occitanus tunetanus OX=6871 PE=1 SV=1	25	3	68	3	1	65	7,5	7,87	44,58	3
6.	Master Protein	P0C298	Toxin Acra III-1 OS=Androctonus crassicauda OX=122909 PE=2 SV=1	28	2	59	1	1	76	8,8	7,11	42,52	2
7.	Master Protein	A0A1E1WVN1	Putative hemocyanin subunit OS=Tityus obscurus OX=1221240 PE=4 SV=1	27	18	62	12	1	628	72,2	6,55	40,66	18
8.	Master Protein	M1JBC0	Sodium channel alpha-toxin Acra4 OS=Androctonus crassicauda OX=122909 PE=1 SV=1	37	4	31	2	1	65	7,1	8,31	34,6	4
9.	Master Protein	A0A1E1WWC5	Putative hemocyanin subunit OS=Tityus obscurus OX=1221240 PE=3 SV=1	17	12	42	9	1	624	71,5	6,19	34,54	12
10.	Master Protein	A0A059UI30	Potassium channel toxin Meg-beta-KTx1 OS=Mesobuthus gibbosus OX=123226 PE=3 SV=1	24	4	38	4	1	91	10,2	8,76	32,05	4
11.	Master Protein	A0A0K0LBX2	Sodium channel blocker AbNaTx2 OS=Androctonus bicolor OX=748906 PE=2 SV=1	10	2	11	1	1	86	9,7	8,31	28,06	2
12.	Master Protein	A0A1E1WVL6	Putative hemocyanin subunit OS=Tityus obscurus OX=1221240 PE=3 SV=1	19	13	54	9	1	637	73,2	6,71	24,77	13
13.	Master Protein	A0A0U4HEU8	Venom protein VP4 OS=Odontobuthus doriae OX=342590 GN=VP4 PE=2 SV=1	15	2	42	1	1	143	16	7,24	22,95	2
14.	Master Protein	P0DPT3	Beta-toxin Am IT OS=Androctonus mauritanicus mauritanicus OX=6860 PE=1 SV=1	25	3	15	3	1	65	7,7	8,62	22,49	3
15.	Master Protein	A0A1E1WVS7	Putative hemocyanin subunit OS=Tityus obscurus OX=1221240 PE=4 SV=1	18	12	34	7	1	632	72,7	5,83	22,07	12

16.	Master Protein	G4V3T9	Neurotoxin BmK AGAP-SYPU2 (Fragment) OS=Mesobuthus martensii OX=34649 PE=1 SV=1	28	3	10	1	1	65	7,2	5,31	20,52	3
17.	Master Protein	P83644	Toxin Lqh4 OS=Leiurus hebraeus OX=6884 PE=1 SV=1	38	4	20	3	1	65	7,2	8,1	19,87	4
18.	Master Protein	M1JMR8	Sodium channel alpha-toxin Acra8 OS=Androctonus crassicauda OX=122909 PE=3 SV=1	30	4	13	2	1	66	7,5	8,29	18,75	4
19.	Master Protein	A0A1E1WW95	Putative hemocyanin subunit OS=Tityus obscurus OX=1221240 PE=4 SV=1	23	14	36	8	1	627	72,1	6,33	17,89	14
20.	Master Protein	A0A0K0LBS4	Venom hyaluronidase (Fragment) OS=Androctonus bicolor OX=748906 PE=2 SV=1	26	3	15	2	1	96	10,8	8,56	12,78	3
21.	Master Protein	A0A0K0LBZ4	Potassium channel blocker AbKTx-6 OS=Androctonus bicolor OX=748906 PE=2 SV=1	10	1	16	1	1	91	10,1	8,4	9,2	1
22.	Master Protein	F8THJ4	CRISP3 (Fragment) OS=Hottentotta judaicus OX=6863 PE=2 SV=1	14	4	15	4	1	192	21,2	8,78	8,2	4
23.	Master Protein	M1JB54	Putative sodium channel alpha-toxin Acra7 OS=Androctonus crassicauda OX=122909 PE=3 SV=1	18	1	4	1	1	67	7,3	7,58	7,75	1
24.	Master Protein	Q7M487	Hemocyanin chain 3C (Fragment) OS=Androctonus australis OX=6858 PE=1 SV=1	50	1	3	1	1	14	1,7	9,85	6,25	1
25.	Master Protein	T1DPC1	CAP-Lyc-1 OS=Lychas buchari OX=1330406 PE=2 SV=1	2	1	3	1	1	422	47	7,53	5,67	1
26.	Master Protein	A0A2I9LNZ2	Endoplasmic reticulum resident protein 44 OS=Centruroides hentzi OX=88313 PE=4 SV=1	10	5	10	5	1	418	48,3	6,35	5,35	5
27.	Master Protein	A0A1E1WWA7	Putative hemocyanin subunit (Fragment) OS=Tityus obscurus OX=1221240 PE=3 SV=1	14	10	18	6	1	618	71,9	6,18	5,25	10
28.	Master Protein	P86100	Hyaluronidase-1 OS=Mesobuthus martensii OX=34649 PE=1 SV=2	10	5	20	1	1	410	47,4	8,16	5,17	5
29.	Master Protein	A0A2I9LNZ4	Fatty acid binding protein OS=Centruroides hentzi OX=88313 PE=3 SV=1	14	2	6	2	1	131	14,8	5,43	4,58	2
30.	Master Protein	A0A1S5QN46	Carboxypeptidase E OS=Tityus serrulatus OX=6887 PE=2 SV=1	5	3	6	2	1	472	53,8	5,58	3,63	3
31.	Master Protein	D5HR53	Anti-insect Ac4 OS=Androctonus crassicauda OX=122909 PE=3 SV=1	10	1	5	1	1	79	8,5	8,31	3,6	1
32.	Master Protein	A0A2I9LNI8	Actin OS=Centruroides hentzi OX=88313 PE=3 SV=1	13	5	7	5	1	376	41,8	5,48	3,58	5
33.	Master Protein	P84646	Alpha-toxin OD1 OS=Odontobuthus doriae OX=342590 PE=1 SV=1	18	2	5	2	1	65	7,2	7,5	3,55	2

34.	Master Protein	A0A0K0LC99	Cellular protein AbCp-54 (Fragment) OS=Androctonus bicolor OX=748906 PE=2 SV=1	38	2	35	1	1	92	10,2	6,29	3,52	2
35.	Master Protein	P01479	Neurotoxin-1'' OS=Androctonus australis OX=6858 PE=1 SV=3	11	1	5	1	1	83	9,1	8,12	3,5	1
36.	Master Protein	F1CJ25	Putative angiotensin converting enzyme (Fragment) OS=Hottentotta judaicus OX=6863 PE=2 SV=1	4	1	3	1	1	255	30,5	6,73	2,47	1
37.	Master Protein	A0A143MGR2	Potassium channel toxin meuK3 OS=Mesobuthus eupeus OX=34648 PE=2 SV=1	15	1	3	1	1	59	6,3	8,7	2,22	1
38.	Master Protein	A0A0K0LBQ2	Pradykinin-potentiating peptide-like peptide Bpp-1 OS=Androctonus bicolor OX=748906 PE=2 SV=1	23	3	6	3	1	73	8,5	10,59	2,16	3
39.	Master Protein	A0A0K0LBR9	Chymotrypsin-like protease ClP-2 (Fragment) OS=Androctonus bicolor OX=748906 PE=2 SV=1	8	1	1	1	1	171	18,9	4,74	2,11	1
40.	Master Protein	F1CJ13	Thioredoxin domain-containing protein (Fragment) OS=Hottentotta judaicus OX=6863 PE=2 SV=1	18	5	11	5	1	319	37,2	5,33	2,1	5
41.	Master Protein	A0AA49K9N2	NaTx OS=Tityus melici OX=3026321 PE=2 SV=1	8	1	1	1	1	85	9,3	8,6	2,08	1
42.	Master Protein	A0A1E1WVR2	Putative transferrin OS=Tityus obscurus OX=1221240 PE=3 SV=1	3	2	4	2	1	711	78,7	5,3	1,98	2
43.	Master Protein	A0A1E1WWG5	Hyaluronidase OS=Tityus obscurus OX=1221240 PE=3 SV=1	11	3	7	2	1	402	46,6	8,72	1,88	3
44.	Master Protein	A0A0U1TYH0	Tetraspanin (Fragment) OS=Isometrus maculatus OX=497827 PE=2 SV=1	4	1	3	1	1	239	26,2	6,51	1,82	1
45.	Master Protein	E4VP53	Chymotrypsin-like protease-4 OS=Mesobuthus eupeus OX=34648 PE=2 SV=1	3	1	3	1	1	270	30,4	5,2	1,81	1
46.	Master Protein	A0A0U1U084	MAM domain-containing protein (Fragment) OS=Isometrus maculatus OX=497827 PE=2 SV=1	4	1	1	1	1	178	19,9	8,78	1,79	1
47.	Master Protein	A0A1E1WVL9	Alpha-amylase (Fragment) OS=Tityus obscurus OX=1221240 PE=3 SV=1	1	1	3	1	1	520	59,5	7,33	1,71	1
48.	Master Protein	A0A2I9LNY0	Elongation factor 1-alpha OS=Centruroides hentzi OX=88313 PE=3 SV=1	2	1	3	1	1	462	51	8,97	1,66	1
49.	Master Protein	A0AA50AAI4	Secretory phospholipase A2 isoform X2 OS=Androctonus crassicauda OX=122909 PE=2 SV=1	12	3	7	3	1	213	25	6,95	1,64	3
50.	Master Protein	A0A088D9S3	Potassium channel blocker pMeKTx8-1 OS=Mesobuthus eupeus OX=34648 PE=2 SV=1	11	1	3	1	1	61	6,9	8,73	1,62	1
51.	Master Protein	U6JTA2	Cysteine protease OS=Tityus serrulatus OX=6887 GN=atg4a PE=2 SV=1	4	1	1	1	1	408	46,7	5,31	0	1

52.	Master Protein	A0A0U1TZ92	14-3-3 protein epsilon (Fragment) OS=Isometrus maculatus OX=497827 PE=2 SV=1	3	1	1	1	1	250	28,7	4,91	0	1
53.	Master Protein	A0A2I9LP41	Heat shock 70 kDa protein OS=Centruroides hentzi OX=88313 PE=3 SV=1	4	2	3	1	1	645	70,7	5,54	0	2
54.	Master Protein	A0A2I9LPF7	Phosphate carrier protein, mitochondrial OS=Centruroides hentzi OX=88313 PE=3 SV=1	2	1	3	1	1	343	38	9,25	0	1
55.	Master Protein	F1CJ20	Putative hemolectin (Fragment) OS=Hottentotta judaicus OX=6863 PE=2 SV=1	2	1	1	1	1	349	40	7,17	0	1
56.	Master Protein	A0A0U1SF04	Peptidase M14 carboxypeptidase A domain-containing protein (Fragment) OS=Isometrus maculatus OX=497827 PE=2 SV=1	8	2	4	1	1	201	23,1	5,14	0	2
57.	Master Protein	P0C2A2	Toxin b subunit alpha (Fragment) OS=Androctonus crassicauda OX=122909 PE=1 SV=1	62	1	9	1	1	26	3,1	4,6	0	1
58.	Master Protein	A0A2I9LP15	peptidylprolyl isomerase OS=Centruroides hentzi OX=88313 PE=4 SV=1	12	2	3	2	1	207	23,6	4,81	0	2
59.	Master Protein	A0A2I9LP74	Peptidylglycine alpha-amidating monooxygenase OS=Centruroides hentzi OX=88313 PE=4 SV=1	2	1	3	1	1	347	39	7,23	0	1
60.	Master Protein	A0A2I9LPI8	Ras-related protein Rab-1A OS=Centruroides hentzi OX=88313 PE=4 SV=1	5	1	1	1	1	206	22,8	5,44	0	1
61.	Master Protein	A0A2I9LNV7	Cysteine-rich secretory protein OS=Centruroides hentzi OX=88313 PE=4 SV=1	2	1	1	1	1	418	47,3	6,81	0	1
62.	Master Protein	A0A0U1S886	lysozyme OS=Isometrus maculatus OX=497827 PE=2 SV=1	13	1	4	1	1	143	16,4	7,47	0	1
63.	Master Protein	A0A1E1WVL0	Ectonucleotide pyrophosphatase/phosphodiesterase OS=Tityus obscurus OX=1221240 PE=4 SV=1	2	1	2	1	1	411	46,8	8,09	0	1
64.	Master Protein	A0A0K0LC76	Cellular protein AbCp-44 (Fragment) OS=Androctonus bicolor OX=748906 PE=2 SV=1	17	2	2	1	1	99	11,3	6,9	0	2
65.	Master Protein	A0A1S5QN36	Xaa-pro aminopeptidase 1c OS=Tityus serrulatus OX=6887 PE=2 SV=1	1	1	3	1	1	667	76,4	5,4	0	1
66.	Master Protein	A0AA49K9R8	phospholipase D (Fragment) OS=Tityus melici OX=3026321 PE=2 SV=1	4	1	1	1	1	410	47,3	8,6	0	1
67.	Master Protein	A0A2I9LPN2	Ubiquitin-ribosomal protein eS31 fusion protein OS=Centruroides hentzi OX=88313 PE=3 SV=1	20	3	5	3	1	156	17,9	9,72	0	3

68.	Master Protein	E4VP21	Chymotrypsin-like protease-1 OS=Mesobuthus eupeus OX=34648 PE=2 SV=1	5	1	3	1	1	264	29,6	5,01	0	1
69.	Master Protein	F1CIX1	Putative alpha-2-macroglobulin (Fragment) OS=Hottentotta judaicus OX=6863 PE=2 SV=1	2	1	2	1	1	266	29,6	4,94	0	1
70.	Master Protein	A0A2I9LP00	Galectin OS=Centruroides hentzi OX=88313 PE=4 SV=1	2	1	3	1	1	465	52,3	6,28	0	1
71.	Master Protein	A0A0K0LC52	Cellular protein AbCp-3 (Fragment) OS=Androctonus bicolor OX=748906 PE=2 SV=1	23	1	1	1	1	64	7,3	11,24	0	1
72.	Master Protein	A0A7S8RFI7	Putative sodium channel toxin Ts37 OS=Tityus serrulatus OX=6887 PE=3 SV=1	8	1	3	1	1	85	9,3	8,27	0	1
73.	Master Protein	Q86BX0	Potassium channel toxin alpha-KTx 15.8 OS=Mesobuthus martensii OX=34649 PE=1 SV=1	15	1	2	1	1	60	6,4	8,7	0	1
74.	Master Protein	A0A2I9LP25	Heat shock 70 kDa protein OS=Centruroides hentzi OX=88313 PE=3 SV=1	6	3	5	2	1	656	72,6	5,34	0	3
75.	Master Protein	A0A0U1TYF8	40S ribosomal protein S19 OS=Isometrus maculatus OX=497827 PE=2 SV=1	5	1	3	1	1	137	15,4	10,35	0	1
76.	Master Protein	A0A2I9LNV5	Cofilin OS=Centruroides hentzi OX=88313 PE=3 SV=1	7	1	2	1	1	148	17	6,57	0	1
77.	Master Protein	P0DL46	Potassium channel toxin alpha-KTx 16.9 OS=Buthus paris OX=1388771 PE=1 SV=1	17	1	2	1	1	36	4,1	8,28	0	1
78.	Master Protein	A0A0U1TZ25	Uncharacterized protein (Fragment) OS=Isometrus maculatus OX=497827 PE=2 SV=1	13	2	5	2	1	93	11	9,76	0	2
79.	Master Protein	A0A0U1SA67	DUF19 domain-containing protein OS=Isometrus maculatus OX=497827 PE=2 SV=1	7	1	1	1	1	229	25,3	5,44	0	1
80.	Master Protein	A0A088D9U2	Potassium channel blocker pMeKTx28-2 OS=Mesobuthus eupeus OX=34648 PE=2 SV=1	16	1	3	1	1	90	10,3	8,65	0	1
81.	Master Protein	A0A059UHJ2	Uncharacterized protein OS=Mesobuthus gibbosus OX=123226 PE=2 SV=1	10	1	3	1	1	147	16,7	4,61	0	1

Metabolite Profile Analysis Results of Scorpion Venom

The analysis of metabolites in a scorpion venom sample was conducted using LC-MS/MS to evaluate free amino acids, carnitine, and acyl carnitine derivatives present in the venom.

Free Amino Acid Profile Results of Scorpion Venom

The profile of free amino acids in the scorpion venom sample is provided in Table 2. The analysis identified high concentrations of amino acids such as lysine, arginine, leucine, and taurine in the venom sample. These findings are consistent with the existing literature.

Table 1. Free Amino Acid Profile of Scorpion Venom

Amino acid	Concentration of Detected Compounds in Venom (nmol/L)
ALANINE	570,8
ARGININE	3439,6
ASPARAGINE	167,5
ASPARTIC ACID	4,3
CITRULLINE	157,2
GLUTAMINE	246,7
GLUTAMIC ACID	498,9
GLYCINE	186,8
HISTIDINE	36,1
LEUCINE	1912,9
ISOLEUCINE	162,3
ALLOISOLEUCINE	13,8
LYSINE	3218,4
METHIONINE	714,1
ORNITHINE	4,8
PHENYLALANINE	959,9
PROLINE	804,7
SERINE	414,4
THREONINE	957,9
TRYPTOPHAN	204,7
TYROSINE	172,2
VALINE	412,1
ALPHAAMINOADIPIC ACID	0,2
ALPHAAMINOPIMELIC ACID	0,0
ANSERINE	2,2
ARGININOSUCCINIC ACID	6,5
ALPHAAMINOBUTYRIC ACID	6,6
BETAAMINOISOBUTYRIC ACID	35,4
GAMMAMINOBUTYRIC ACID	224,2
BETA-ALANINE	6,7
SARCOSINE	22,6
CYSTATHIONINE	1,3
THIAPROLINE	25,3
1-METHYLHISTIDINE	0,8
3-METHYLHISTIDINE	1,7

HYDROXYLYSINE	4,7
HYDROXYPROLINE	690,9
CYSTINE	0,4
HOMOCYSTINE	0,0
SEROTONIN	0,1
HISTAMINE	2,2
ETANOLAMINE	549,3
PHOSPHOETANOLAMINE	240,1
5-OH-TRP	18,2
TAURINE	3625,1

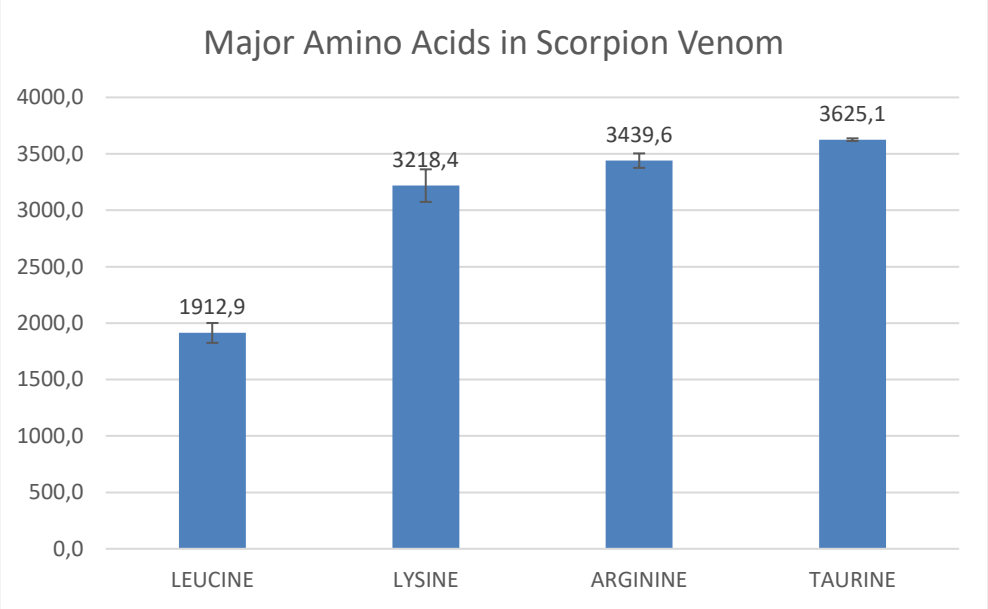

Fig 2. Graphical Representation of Free Amino Acid Profile in Scorpion Venom as Analyzed by LC-MS/MS (nmol/L))

Free Carnitine and Acyl Carnitine Profile Results in Scorpion Venom

The profile of carnitine and acyl carnitine derivatives contained in the venom sample is presented in Table 3. According to LC-MS/MS results, the venom contains C0 (free carnitine) and C2 (acetyl carnitine) among other free carnitine and acyl carnitine derivatives.

Table 3. Free Carnitine and Acyl Carnitine Profile in Scorpion Venom

Carnitine Name	Concentration Detected in Venom (µmol/L)
C0 (serbest karnitin)	3,3
C2 (asetil karnitin)	1,1
C3 (Propiyonil Karnitin)	0,0
C4 (bütiril karnitin)	0,0
C4DC (metil malonil karnitin)	0,0
C5 (isoyaleril karnitin)	0,0
C5:1 (tiglil karnitin)	0,0
C5OH (isoyaleril karnitin)	0,0
C5DC (glutaril karnitin)	0,0
C6 (hekzanoil karnitin)	0,1
C6DC (adipil karnitin)	0,0
C8 (oktanoil karnitin)	0,0
C8:1(oktenoil karnitin)	0,0
C8DC(suberil karnitin)	0,0
C10 (dekanoil karnitin)	0,0
C10:1 (dekenoil karnitin)	0,0
C10DC (sebasil karnitin)	0,2
C12 (dodecanoil karnitin)	0,0
C14 (miyristoil karnitin)	0,0
C14:1	0,0
C14:2	0,0
C16 (palmitoil karnitin)	0,0
C16:1 (palmitoleil karnitin)	0,0
C18 (steraoil karnitin)	0,0
C18:1(oleil karnitin)	0,0
C18:2 (linoleil karnitin)	0,0
C18:1 OH (hidroksiOleil karnitin)	0,0

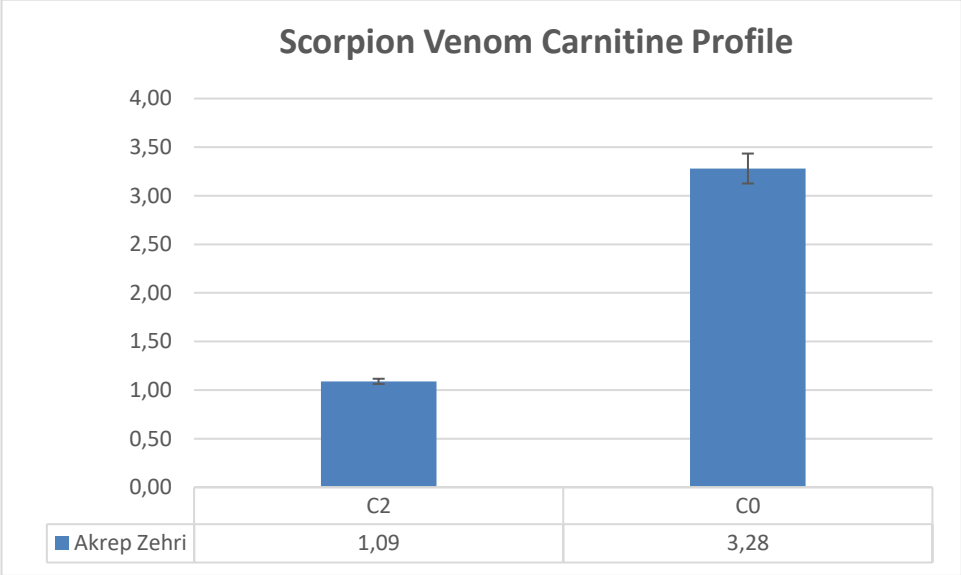

Fig 9. Graphical Representation of Free Carnitine and Acyl Carnitine Profile in Scorpion Venom as Analyzed by LC-MS/MS (μmol/L).

Analysis of the Cytotoxic Effects of Scorpion Venom on Normal Cells

The cytotoxic effects of scorpion venom on various normal cell types were investigated using the MTT assay method. IC50 values were determined after examining the dose-dependent changes in cell viability. The venom was applied to the cells at concentrations of 0, 2.5, 5, 10, 25, 50, 100, and 200 µg/ml, and its cytotoxic effects were assessed 24 hours later. The viability of control group cells (0µM), which did not receive venom, was considered 100% at all time points, and the viability of other samples was calculated proportionally based on absorbance values using Graph Pad Prism 9.5.0. High doses of scorpion venom were found to have significant cytotoxic effects, as evidenced by the IC_{50} values.

CELL LINE	CRL-4010	BEAS-2B	HUVEC	HEK-293	PNT1-A
IC50 (µg/ml)	120,3	219,2	122,3	124,8	165,3

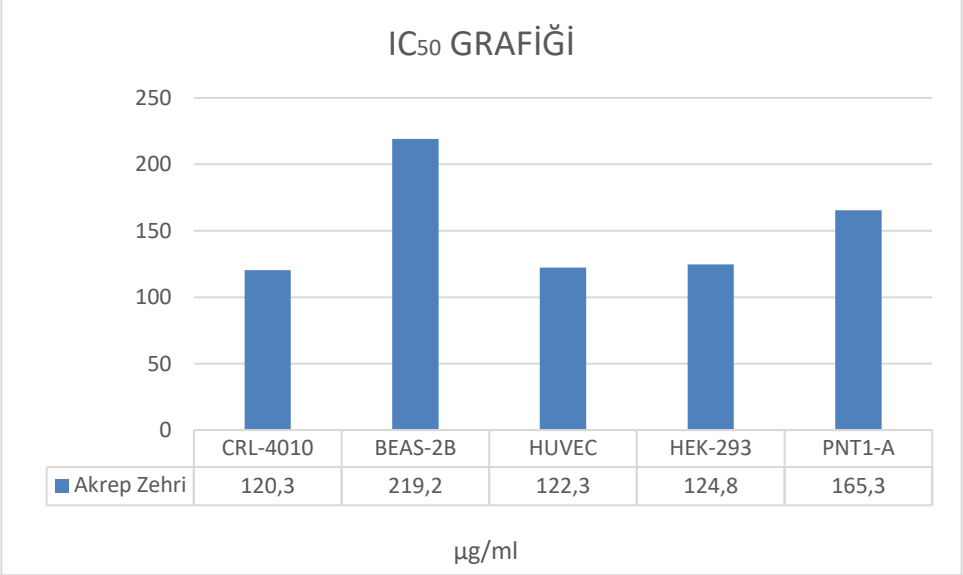

Fig 10. Graphical Representation of IC_{50} Values of Scorpion Venom on Normal Cell Lines