ORAL ABSTRACTS

PLENARIES

001: Snake-bite clinical aspects: challenges and opportunities

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Snake-bite's time has come!

Snake-bite has been re-admitted to WHO's list of Neglected Tropical Diseases, now with category A prioritisation, has the backing of Kofi Anand's Foundation, the Wellcome Trust and the Lillian Lincoln Foundation, and was recently supported by a World Health Assembly resolution. There now exists the best ever opportunity for seeking funding for clinical research on this neglected, denied and abandoned disease.

Epidemiology

Reliable estimates of national morbidity and mortality depend on community-based studies that avoid the inaccuracies and omissions of hospital-based statistics. These have been achieved only in India, Bangladesh, and Sri Lanka. Associated physical and mental morbidity has not been properly assessed. Expertly-designed, community-based national surveys of bites, envenomings and sequelae are needed for countries where snake-bite is an important public health issue.

Clinical trials of snake-bites

A fundamental requirement for all clinical studies of snake-bite is species diagnosis, based on expert identification of the dead/living specimen or a photographic image of the animal responsible, or laboratory confirmation by ELISA or PCR. Unfortunately, immunodiagnosis as a vital research tool has been established in very few countries. This must be remedied.

Antivenoms

WHO has reviewed all aspects of antivenom production and is carrying out critical inspections and pre-clinical assessments of antivenom manufacturers and their products. Most antivenoms, have never been subject to formal clinical trials. Widespread uncertainties about indications, initial and subsequent dosage, effectiveness and safety that can be resolved only by formal Phase I-IV clinical trials as recommended by WHO. There is an urgent need to establish clinical platforms for these trials in countries that have the necessary numbers of cases and clinical resources.

002: Toxin-resolved venom proteomes: a challenge in evolutionary and translational venomics

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Venoms are integrated phenotypes used by a wide range of organisms for predatory and defensive purposes. The study of venoms is of great interest in diverse fields, such as evolutionary ecology and biotechnology. On the other hand, legitimate snake bites caused to humans in their share natural environment is a serious neglected public health issue that disproportionaly affects the most impoverished and geopolitically disadvantaged rural communities of tropical and subtropical countries. This occupational and environmental "disease of poverty" affects 1.8-2.7 million people worldwide each year, causing 85-138.000 deaths, maiming 400.000 victims, and leaving an indeterminate number of survivors with psychological sequelae and other posttraumatic stress disorders. Toxins bearing the highest prey incapacitation activity are often also the most medically important molecules in the context of a human envenoming. Ecological and translational venomics are two sides of the same coin. Thus, understanding the complexity of venoms and their locus-resolved toxicological profiles can shed light on the mutually enlightening relationship between evolutionary and clinical toxinology to identify those toxins that should be neutralized to reverse the pathological effects of venom. Omics technologies have contributed to understanding the evolutionary mechanisms that molded snake venoms to their present-day structural and functional variability lanscape. The recent implementation of top-down MS and **a**bsolute quantification of intact proteins by elemental mass spectrometry promise to represent a quantitative leap in the transition to achieve toxin-resolved venom proteomes**.** The quantitative analysis of an antivenom's ability to recognize and neutralize the toxins present in medically relevant snake venoms represents the cornerstone on which its clinical qualification should be based. The recently developed immunoaffinity chromatography-based third-generation antivenomics (3GA) platform allows the determination of the toxin-binding capacity of antivenom towards the individual venom toxins and to quantify the percentage of clinically effective antibodies present in the antivenom. These parameters assist *in vivo* neutralization assays in the assessment of the clinical landscape of homologous and heterologogous antivenoms in a phylogeographical context. Integrating toxin-

resolved venomics, 3G antivenomics, and toxicovenomics into a single platform represents a challenge in evolutionary and translational venomics.

003: Lessons for clinical toxinology from the Myanmar snakebite project

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Clinical toxinology is a specialised field of medical expertise encompassing toxin-induced-diseases (TIDs), excluding bacterial toxin diseases. It includes a wide variety of envenomings and poisonings, caused by toxins produced by animals, plants and mushrooms, including minute organisms such as dinoflagellates. Snakebite arguably enjoys the highest profile of TIDs and was recently designated a Neglected Tropical Disease by WHO, a decision which may allow substantially increased funding to be applied to tackling snakebite globally. Understanding how clinical toxinology can contribute towards such a global effort is important in maximising the effectiveness of that effort. The Myanmar Snakebite Project is an Australian Government DFAT-funded foreign aid project, commenced in 2014 and concluding in 2018. The core aim was improvement in outcomes for snakebite patients in Myanmar, which has a historically high snakebite burden, with snakebite a poverty trap for poor rural communities. The Project was conceived as a holistic approach, covering aspects of prevention, public education, epidemiologic analysis, with both community-based surveys and hospital-based case data collection, training of health workers at all levels, provision of training and resource materials including treatment protocols, improvement in local antivenom production, both in quantity and quality, and improved distribution and availability of antivenom. To achieve this an international team of experts was assembled and interfaced with Myanmar colleagues to enshrine a fully collaborative approach, aiming for full local ownership of developments and solutions to ensure sustainability. Current indications are that both on the Ministry of Industry side, involved with antivenom production, and now on the Ministry of Health side, involved with most other aspects of delivery of care to snakebite patients, there has been a progressive and successful local engagement and intention to maintain and further improve outcomes achieved to date, which argues towards sustainability. This has been achieved by continued development of positive personal interactions between Project members and their local counterparts and recognition that all concerned is learning from this Project, not just applying previous knowledge. This has been a journey which hopefully will continue. While some

aid funds purchased equipment, this represented only a small part of Project expenditure and effort; it has been the human interaction component that has driven success and willingness to build on existing structures and knowledge within Myanmar. While antivenom is a key part of the snakebite treatment process, it remains only one part, and should not dominate aid efforts, including through clinical toxinology, to improve outcomes for envenomed patients.

004: The Myanmar Snakebite Project; an initial analysis of 3880 cases of snakebite.

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The Myanmar Snakebite Project (MSP), an Australian Government DFAT-funded foreign aid project, 2014-2018, has a principal aim of improving outcomes for snakebite patients in Myanmar, a country with a significant snakebite case load. As part of the MSP a database was created to collect snakebite case records, used primarily to guide the Myanmar Ministry of Health response to management of snakebite as part of the Myanmar Snakebite Project (MSP).

Trained MSP local medical officers collected data on snakebite patients presenting to selected hospitals. Data was entered on hard copy forms and later transferred to a purpose-built FileMakerPro database, for analysis. Patient consent was received for all included cases. Where possible dead snakes brought in by patients were preserved and retained for expert identification.

Data collection commenced in late 2015 and, for the purpose of this abstract, extends to July 2018. In this 2.5 year period 3880 cases were entered into the database, 3251 from the Mandalay region of which 2313 were from Mandalay General Hospital, and 629 from Yangon (fewer hospitals and a shorter collection period of 18 months). In the Mandalay region, of 2646 cases where a snake ID

was provided, 78% were Russell's viper (RV), 12% were "green snake" (GS; mostly green pit viper, GPV), with only 2.8% cobra (Co), <1% krait. Most of the cobra bites where a dead snake could be identified were by Naja mandalayensis which caused predominantly local effects rather than neurotoxicity. The case fatality ratio in this region was 8.1%, almost all cases following RV bite, though with a single death from king cobra bite. Amongst GS bites, 20% had coagulopathy, 9 had evidence of acute kidney injury (AKI), 1 requiring dialysis. Amongst RV bites 62% had coagulopathy, 52% had AKI, 26% required dialysis and 11% died. More limited data from Yangon indicated a similar preponderance of RV bites (83%) and case fatality ratio (8.9%), but because cases were only collected from 2 hospitals which manage mainly more severe cases, this may be unrepresentative and artificially exclude most bites by other species.

Snakebite causes a significant hospital workload in Myanmar, particularly RV which is the major cause of AKI and snakebite fatality. Data collected by this project is assisting development of health system strategies to better manage snakebite in Myanmar. GPV bites are a significant problem numerically and can cause diagnostic confusion with RV bites, with implications for treatment and antivenom development.

005: Improving the transition from toxins to medicines

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There are examples of research on toxins leading to new medicines in clinical use. They range from the poisonous plant alkaloids like tubocurarine that were the prototypes for synthetic analogues with improved safety profiles, through compounds such as captopril that were modelled on short peptides from snake venoms, to actual venom peptides (like ziconotide) and protein toxins (notably various forms of botulinum toxins). Toxins as a route to drug discovery have attracted the enthusiastic attention of many toxinologists. However, the response from pharmaceutical development companies has been welcoming. Although many research projects aspire to drug discovery, very few enter the seriously competitive stage of preclinical assessment, let alone clinical trials. The reasons why this should be are explored in this presentation.

Primarily, the research can be regarded as starting in the wrong place: in most cases, the activity of the toxin is discovered and then an application is looked for. In other words, there is 'technology push' rather than 'market (or therapeutic need) pull'. With the toxin researchers anxiously trying to find an end use for their toxin, they may not fully appreciate the competition from other, often more conventional, solutions. Can the toxin truly address an unmet therapeutic need in a way that other approaches cannot do? If so, can the toxin be demonstrated to have an acceptable side-effect profile? Although many toxins offer an attractive combination of high potency and selectivity, it is rare for diseases to be caused by defects in single molecular targets that only occur in the affected tissue. Beyond that, does the toxin have acceptable pharmaceutical characteristics (e.g., stability, absorption, metabolism)? Is the toxin able to be economically manufactured in sufficient quantity?

Successful drug discovery is a difficult business, with success often involving large amounts of good luck. Novel biological actions of toxins can undoubtedly point the way to new therapeutics, but researchers need to be more aware of the obvious pitfalls along the way.

006: The potential of recombinant antibody technology in venom therapeutics

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The display of antibodies on the surface of filamentous phage has proven to be a powerful technology in antibody generation in both research and drug discovery. It becomes possible to create antibody libraries of 40 billion clones and to isolate human antibody genes encoding desired properties. The presentation will review the use of antibody phage display to isolate antibodies and will exemplify the generation of neutralising antibodies generated against fractionated venom of the black mamba.

Venoms components have the potential for good as well as harm. A multitude of venomous animals block ion channels using small cysteine-rich peptides in defence or predation. However, such naturally occurring "knottin" blockers of ion channels often suffer from manufacturing difficulties, short half-lives and a lack of specificity. Using phage display we have developed a novel molecular fusion format wherein naturally occurring cysteine-rich peptides are inserted into peripheral CDR loops of an antibody while retaining the folding and function of both molecules. We have demonstrated functional insertion of multiple cysteine-rich peptides which block the voltage-

gated potassium channel, Kv1.3 and the acid sensing ion channel ASIC1a. The modular nature of the KnotBody binding surface and the amenability of this format to phage display technology will facilitate further optimisation of potency and selectivity of ion channel blockade by engineering both knottin and antibody loop sequences.

007: Venom components with insecticidal activity found in major animal phyla

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During countless years of evolution, venomous animals in major phyla have developed sophisticated strategies to tailor their venom peptide components and deploy them successfully with offensive and defensive purposes. These peptides have drawn considerable attention because of their surprising potency and selectivity, enabling a promising biodiversity-based and biotechnological-type of research for the development of novel bioactive components in the field of pharmaceutical and agricultural applications.

Pest insect species are a threat to humans as they can destroy crops and serve as vectors for a wide range of diseases including malaria and dengue. Human health risks and ecological concerns in combination with the development of insecticide resistance, linked to the use of chemical insecticides, have created a need for an alternative approach of insect pest control.

For the discovery of novel insecticides, the phylum of Arthropoda is extremely interesting as it contains numerous venomous species found in different subphyla: Arachnida, with spiders and scorpions; Myriapoda, with centi- and millipedes; Crustacea, with shrimps and crabs, and Hexapoda, with insects.

Recent research has revealed that also other phyla such as the Nemertea (with ribbon worms) and Cnidaria (with sea anemones and jellyfishes) contain species with surprising peptide structures in their venoms and unprecedented insecticidal action. Some novel examples of peptidyl toxins, both native and synthetically modified (e.g. cyclized), targeting the invertebrate voltage-gated sodium channel (Nav) at the low nM level, using different peptide scaffolds, will be presented and discussed.

008: Applying systems biology and genomic manipulation approaches for characterizing the dynamics and complexity of venom production in a cnidarian

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The starlet sea anemone *Nematostella vectensis*, a model cnidarian, offers unprecedented research tools unavailable for most other venomous animals. These tools include unlimited access to all developmental stages, gene knockdowns and knockouts and introduction of reporter genes into the genome. In our studies we harness these valuable tools to the study of venom. The appearance of stinging cells and ectodermal glands cells in very early life stages of *Nematostella* as well as our transcriptomic, proteomic and gene localization studies reveal that venom is synthesized already in eggs, early embryos and larvae and that different toxins are expressed in distinct life stages and diverse cell populations. These findings suggest a much more complex and dynamic venom landscape than initially appreciated. To fully map this landscape we are generating transgenic animals that express fluorescent reporters fused to toxins under the control of toxin genetic regulatory elements enabling high-resolution toxin localization. Further, state-of-the-art genetic engineering approaches such as the CRISPR/Cas9 system enable targeted manipulation of the sea anemone genome and raise the possibility to test the physiological and ecological importance of toxin-coding genes. These approaches expand our understanding of the origins of toxin-producing cells in Cnidaria and bring together biological disciplines that rarely meet, such as developmental biology, toxinology and ecology to enable a truly panoramic view of the dynamics of venom production as well as its evolutionary history.

KEYNOTE LECTURES

009: Mass spectrometry imaging as a tool for providing a better understanding of venom biology

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Despite decades of research, we still have a poor understanding of the biological functions of most toxins in animal venoms. Part of this poor understanding is due to a lack of knowledge about the way venoms are produced and stored in the venom gland and associated structures, mainly arising from the limited information that is gained by targeted imaging approaches. Mass spectrometry imaging (MSI) enables detection of compounds directly off tissue sections and other flat surfaces. Particularly MALDI imaging is gaining popularity for non-targeted imaging of compounds from tissue, and has proved invaluable in addressing the shortcoming of other imaging techniques in imaging the spatial distribution of novel compounds. It is also an ideal technique for investigating the spatial distributions of toxins in venom glands. In combination with traditional transcriptomic, proteomic, histological techniques, and other MSI modalities such as ICP-MSI, MALDI imaging of venom gland sections has shed new light on the biology of venom production and secretion. This has revealed a surprising spatial heterogeneity of toxin production in a range of venomous animals, and helped us put the evolution of venoms and toxins into both a functional and morphological context. It has also highlighted venom glands as excellent model systems for development of new MSI-based methods with novel biomedical applications, such as "functional MSI" which allows imaging biological activity across tissues. This combination of venomics, MSI, and functional MSI, is likely to generate new insight into the biology of animal venoms, including snakes.

010: Three finger toxins: recent findings

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Three-finger toxins (TFT) comprise one of the largest families of snake toxins; they are the principal and the most toxic components of venoms from Elapids. The first TFT α-bungarotoxin (α-Bgt) has been discovered almost fifty years ago and so far is widely used as a specific marker of α7 and muscle-type nicotinic acetylcholine receptors (nAChR). The discovery of new activities for both the new and the well known TFTs is a recent trend. We showed that TFT α-cobratoxin completely blocked GABA-induced currents in the corresponding receptor expressed in *Xenopus* oocytes. The GABA-A receptor was also inhibited by some other TFTs with mixed competitive and noncompetitive action. Further, a new TFT nakoroxin was isolated from the cobra venom, and its complete amino acid sequence was established. It belongs to the group of "orphan" toxins, data on the biological activity of which are practically absent. Nakoroxin shows no cytotoxicity and does not inhibit the binding of α-Bgt to nAChRs of muscle and α7 types. However, it potentiates the binding of α-Bgt to the acetylcholine-binding protein from *Lymnaea stagnalis*. This is the first toxin with such an unusual property. Nonconventional TFT BMLCL was isolated from *Bungarus multicinctus* venom, and its interaction with different subtypes of nAChR was studied. It was found that BMLCL is able to interact efficiently with both α7 and muscle type nAChRs. Genes encoding two TFTs, namely TFT-AF and TFT-VN, nucleotide sequences for which were earlier determined by cloning cDNA from venom glands of vipers *Azemiops feae* and *Vipera nikolskii*, respectively, were expressed in *E. coli* cells. The study of biological activity of these toxins by electrophysiological techniques, calcium imaging, and radioligand analysis showed that the viper TFTs are antagonists of nAChRs of neuronal and muscle type.

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011: Tetrodotoxin in newts - Endogenous or exogenous origin?

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Tetrodotoxin (TTX), the highly potent neurotoxin blocking voltage-gated sodium channels, occurs in a wide range of marine as well as terrestrial animals.

In amphibians, the toxin is present in Asian newts of the genus Pachytriton, Laotriton, Paramesotriton, Cynops, and in New-World newts of the genus Taricha and Notophthalmus. In these newts, high intraspecific variability ranging from zero to high TTX-levels are observed. However, the biological origin of the toxin is still a matter of discussion. Whether it is synthesized by the newts or it is sequestered from exogenous sources is an open question.

Breeding experiments of wild-caught Cynops orientalis from China, C. pyrrhogaster from Japan, Notophthalmus viridescens from Pennsylvania, USA, and Taricha granulosa from British Columbia, Canada, showed that larvae of these TTX-bearing newts were entirely toxin-free and, when raised in captivity, juveniles remained non-toxic even after more than one year. Whether the newts generally lack the capability of TTX-biosynthesis, as these experiments may suggest, or they sequester the toxin from sources in their environment (ponds with a mud bottom, pools of flowing streams), or whether the conditions in captivity are not appropriate to trigger or support toxin synthesis, needs further in-depth studies.

012: The impact of site-specific positive selection on the structurally and functionally important parts of the snake venom Kunitz/BPTI protein family

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S1 family of serine peptidases is the largest family of peptidases. They are specifically inhibited by the Kunitz/BPTI inhibitors. Kunitz domain is characterized by the compact 3D structure with the most important inhibitory loops for the inhibition of S1 peptidases. We analysed the action of site-specific positive selection and its impact on the structurally and functionally important parts of the snake venom Kunitz/BPTI family of proteins. By using numerous models we demonstrated the presence of large numbers of site-specific positively selected sites that can reach between 30–50% of the Kunitz domain. The mapping of the positively selected sites on the 3D model of Kunitz/BPTI inhibitors has shown that these sites are located in the inhibitory loops 1 and 2, but also

in the Kunitz scaffold. Amino acid replacements have been found exclusively on the surface, and the vast majority of replacements are causing the change of the charge. The consequence of these replacements is the change in the electrostatic potential on the surface of the Kunitz/BPTI proteins that may play an important role in the precise targeting of these inhibitors into the active site of S1 family of serine peptidases.

013: Incidence of snakebites and medically relevant snakes in different regions in Laos and Vietnam

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Incidence of snakebites is largely unknown in Lao PDR and Vietnam. Community-based surveys were performed in Savannakhet province, southern Laos, Thua Thien Hue province in central Vietnam and Can Tho Municipality in the Mekong Delta of southern Vietnam in order to estimate the incidence of snakebites in these regions. Evaluation of hospital admissions of snakebite patients in Savannakhet province, Vientiane capital city and Vientiane province in Lao PDR and in Hue and Can Tho municipality in Vietnam provided information about medically relevant snakes in these regions.

Incidence of snakebites is high in Savannakhet province with up to 1105 snakebites per 100,000 persons per year. Malayan pit vipers, green pit vipers, Indochinese cobras and Malayan kraits were responsible for 40%, 30%, 25% and 5% of snakebites respectively. In Thua Thien Hue province in central Vietnam and Can Tho municipality in South Vietnam incidence was calculated at 58 and 40 snakebites per 100,000 persons per year respectively. Green pit vipers and cobras caused the majority of snakebites in both regions.

The significantly lower incidence of snakebites in Central and Southwest Vietnam compared to Lao PDR can be explained by advanced mechanization in agriculture, a lower poverty rate, a

different snake fauna and the exploitation of snakes for food, snake wine and traditional healing practices. Furthermore the increasing urbanization and cultivation of land for commercial purpose in this densely populated country most likely deprive snakes of their habitat.

014: Gomesin inhibits melanoma growth by manipulating key signaling cascades that control cell death and proliferation

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Consistent with their diverse pharmacology, peptides derived from venomous animals have been developed as drugs to treat disorders as diverse as hypertension, diabetes and chronic pain. Melanoma has a poor prognosis due in part to its metastatic capacity, warranting further development of novel targeted therapies. This prompted us to examine the anti-melanoma activity of the spider peptides gomesin (AgGom) and a gomesin-like homolog (HiGom). AgGom and HiGom dose-dependently reduced the viability and proliferation of melanoma cells whereas it had no deleterious effects on non-transformed neonatal foreskin fibroblasts. Concordantly, gomesin-treated

melanoma cells showed a reduced G0/G1 cell population. AgGom and HiGom compromised proliferation of melanoma cells via activation of the p53/p21 cell cycle check-point axis and the Hippo signalling cascade, together with attenuation of the MAP kinase pathway. We show that both gomesin peptides exhibit antitumoral activity in melanoma AVATAR-zebrafish xenograft tumors and that HiGom also reduces tumour progression in a melanoma xenograft mouse model. Taken together, our data highlight the potential of gomesin for development as a novel melanoma-targeted therapy.

015: The first intrinsic tenase complex inhibitor with serine protease structure offers a new perspective in anticoagulant therapy

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Components of the intrinsic blood coagulation pathway, among them FVIIIa, have been recognized as suitable therapeutic targets to treat venous thromboembolism, pathological process behind two very serious cardiovascular diseases, deep vein thrombosis and pulmonary embolism. Here we describe a unique glycoprotein from the nose-horned viper (*Vipera ammodytes ammodytes*) venom, *Vaa*SPH-1, structurally a serine protease but without an enzymatic activity and expressing potent anticoagulant action in human blood. We demonstrated that one of its targets in the blood coagulation system is FVIIIa of the intrinsic tenase complex, where it antagonizes the binding of FIXa. Anticoagulants with such characteristics are intensively sought, as they would be much safer for medical application as the contemporary drugs, which frequently induce excessive bleeding and

other complications. *Vaa*SPH-1 is unlikely to be orally available for chronic usage as it has molecular mass of 35 kDa. However, it represents a very promising template to design low molecular mass FVIIIa-directed anticoagulant substances, based on structural features of the interaction surface between *Vaa*SPH-1 and FVIIIa. To this end, we constructed a three-dimensional model of *Vaa*SPH-1 bound to FVIIIa. The model exposes the 157–loop and the preceding α-helix as the most appropriate structural elements of *Vaa*SPH-1 to be considered as a guideline to synthesize small FVIIIa-binding molecules, potential new generation of anticoagulants.

016: Subtle substitutions in toxins: Design of natriuretic peptide analogues for personalized care of heart failure patients

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Natriuretic peptides (NPs) are potent vasoactive hormones, which maintain pressure–volume homoeostasis. All three isoforms of mammalian NPs, namely ANP, BNP and CNP, have a conserved 17-residue ring but with highly variable C-terminal extensions. These peptides confer their functions through binding to three membrane-bound NP receptors (NPRs). ANP and BNP bind to NPR-A, whereas CNP binds to NPR-B, both guanylyl cyclase (GC) linked receptors. NPR-A and NPR-B undergo conformational change upon respective NP binding and lead to the production of intracellular cGMP. Snake venom NPs, although have the conserved NP-ring, exhibit distinct biological activity compared with mammalian NPs due to subtle changes in their sequences. We identified a new NP from krait venom (KNP), with an unusual 38-residue long C-terminal tail, which has a propensity to form an α-helix. Deletion mutant studies have revealed the presence of two pharmacophores in KNP, namely Ring and Helix. Both these functional segments induce vasodilation, but through orthogonal pathways. Ring, like a classical NP, elevates intracellular cGMP levels through activation of NPR-A with a 10-fold lower potency compared to ANP, while Helix uses NO-dependent mechanisms. Interestingly, despite subtle substitutions, Ring shows only vasodilation in contrast to mammalian NPs, which induce both vasodilation and diuresis. By systematic structure-function studies of the Ring peptide, we identified the residues responsible for the vasodilatory and diuretic functions. Using this new knowledge, we developed two classes of

human natriuretic peptide analogues (NPAs); one group of NPAs with only vasodilatory effects without diuretic function and the second group with only diuretic effects without vasodilatory function. Such distinct classes of NPAs will be useful in the treatment of distinct classes of ADHF (acute decompensated heart failure) patients.

017: Molecular actions underlying the biomedical applications of recombinant variants of botulinum neurotoxins

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Botulinum neurotoxin type A (BoNT/A)-haemagglutinin complex from *Clostridium botulinum* is a selective, prolonged inhibitor of neurotransmitter release used to treat several conditions arising from hyper-activity of cholinergic nerves innervating muscles or secretory glands. Also, the toxin complex alleviates chronic headache in certain patients and nocifensive behaviour in pain models. Although BoNT/A blocks K^+- elicited exocytosis from cultured sensory neurons of a pain mediator, calcitonin gene-related peptide (CGRP), its release triggered by capsaicin activation of a transient receptor potential channel (TRP) /V1 is insensitive. However, a chimera (LC/E-BoNT/A) inhibits this release evoked by all stimuli tested, and displays analgesic activity in a preclinical model of neuropathic pain. As this requires a much lower dose than the concentration needed for lowering the CGRP release, its effects on the surface trafficking of TRP/V1 and /A1 channels were investigated. Their plasmalemmal content on sensory neurons becomes elevated in response to inflammatory cytokines, so we assessed its toxin susceptibility. Immuno-labelling of neurons from rat dorsal root ganglia revealed that the enhancement of TRP/V1 and A1 by the cytokine, TNFα, is decreased by pM LC/E-BoNT/A, which also diminished the TNFα-induced increments in the TRP/V1 and /A1 currents, as measured by patch-clamp and Ca^{2+} influx. Significantly, only the cytokine-augmented, and not the resting, trafficking of the functional channels were affected by the chimera, accompanied by the production of /E- and /A-cleaved SNAP-25. Whilst these findings reaffirm that the cytokine-elevated surface trafficking of the two transducers is SNAP-25 dependent, its level of contribution to alleviating pain *in vivo* remains to be determined.

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018: Cocktails of human monoclonal IgG antibodies capable of neutralizing dendrotoxin-mediated neurotoxicity of black mamba venom *in vivo*

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The African black mamba (*Dendroaspis polylepis*) is one of the most feared snakes in the world due to its potent venom, which is comprised of fast-acting neurotoxic venom components, including dendrotoxins and α-neurotoxins. Timely treatment with effective antivenom is essential against envenoming by *D. polylepis*, as untreated victims experience a very high fatality rate. However, existing animal plasma-derived antivenoms are scarce on the African continent and present a number of drawbacks due to their heterologous nature.

Here, we report the development and *in vivo* assessment of an experimental recombinant antivenom against dendrotoxin-mediated neurotoxicity of *D. polylepis* venom. By combining a toxicovenomics and phage display approach, we successfully developed a suite of fully human immunoglobulin G (IgG) monoclonal antibodies that can neutralize *D. polylepis* dendrotoxins in mice. By selecting the most promising of these human IgGs to formulate defined oligoclonal IgG cocktails, we demonstrated that dendrotoxin-mediated neurotoxicity of *D. polylepis* whole venom can be completely neutralized in an experimental mouse model using intracerebroventricular injection. This report represents the first discovery of fully human monoclonal IgGs against animal toxins and the first design and assessment of a recombinant antivenom based on oligoclonal human IgG mixtures against experimental snakebite envenoming.

019: Challenges in antivenom downstream processing efficiency estimation

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The hyperimmune horse plasma (HHP), prepared by active immunisation of horses with an antigen of interest, is the most common starting material for antitoxin (animal antibody-based therapeutics) production. Precise IgG quantification in plasma is a prerequisite for accurate estimation of the purification process efficiency. Although immunoglobulins from HHP have been purified for more than a century, we still lack in vitro method for precise and accurate determination of IgG content in HHP. In such situation, the purification process efficiency has been assessed by antibody activity measurements, performed in vivo.

The development of a precise and accurate in vitro immunoassay for IgG quantification in HHP will be presented. It is based on a novel concept of sample-specific correction of immunoassay results. We discovered that any difference in composition of IgG population between the standard and the sample, with respect to both IgG subclass distribution and antigen-specific IgG content, leads to inaccurate IgG quantification. We demonstrated that caprylic acid precipitation as the method for IgG isolation from horse plasma renders the composition of IgG population unchanged and was used to prepare internal, sample-specific reference IgG for each plasma sample, which was tested simultaneously to a respective plasma sample.

The application of the novel method for the estimation of the active principle yield in the process of IgG purification from HHP will be demonstrated. Also, the impact of subclass changes due to different purification processes on the quality of the final product will be discussed.

020: Cobra Venom Factor: A Lead Venom Component for the Development of a Biologic for the Treatment of Complement-mediated Diseases

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Cobra Venom Factor (CVF) is the anti-complementary protein in cobra venom. It is a structural and functional analogue of complement component C3, forming a bimolecular complex with complement Factor B, an enzyme that cleaves C3 (C3 convertase). The convertase formed with CVF is, in contrast to C3b, stable and resistant to inactivation by regulatory proteins, leading to depletion of plasma complement. Whereas complement depletion is not toxic, exhaustive complement activation by the stable convertase at the envenomation site aids the toxic venom components to enter the bloodstream.

Complement is also part of the pathogenesis of many diseases. Pharmacological inhibition of complement is therefore a desired goal. We created chimeric proteins of human C3 with CVF by exchanging the functionally important region in human C3 with the corresponding homologous region from CVF. These chemeri proteins (called humanized CVF (hCVF)) are human C3 derivatives with the CVF-specific function of forming a stable convertase, causing complement inhibition by consumption. Beneficial therapeutic effects of complement depletion have been shown in multiple preclinical models of disease, including myocardial and gastrointestinal reperfusion injury, age-related macular degeneration, myasthenia gravis, paroxysmal nocturnal hemoglobinuria (PNH), and others. No toxic side effects were observed in any disease model or after intra-arterial administration to healthy primates. Moreover, although hCVF is mildly immunogenic in mice - in contrast to natural CVF which is highly immunogenic -, no neutralizing antibodies were formed in mice, even after repeated administration of hCVF. Recently, we created new hCVF clones for consistent recombinant expression (referred to as iC3).

Our results show that complement depletion with hCVF represents a promising therapeutic concept for complement-mediated diseases, and that venom components can serve as valuable lead compounds for drug development.

021: Combined animal and human data in support of effectiveness of antivenom against *M. fulvius* neurotoxicity

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Under some circumstances, it is neither feasible nor ethical to conduct prospective controlled clinical trials of antivenom against neurotoxic elapid envenomation. One such case involves coral snake envenomation in the USA. We studied the effectiveness of equine $F(ab')_2$ anti-Micrurus *fulvius* antivenom by combining an historically controlled, prospective human study with a novel large animal model.

Pharmacokinetic parameters of venom uptake, at subcutaneous doses of *M. fulvius* venom comparable to those expected in bitten humans, were established in Suffolk-Pelibuey hybrid sheep. Sheep with and without thoracic duct cannulation were compared, establishing that absorption from subcutaneous tissue was the rate-limiting step for venom bioavailability. A standardized rescue dose of intravenous antivenom was then applied using the same model, 2 hours after venom injection. These experiments showed that intravenous antivenom effectively binds venom in the bloodstream, but that residual unbound venom in local tissue and lymph has the potential to result in ongoing systemic uptake and distribution, hours later.

We used published reports of *M. fulvius* bites to establish an historic human mortality rate of 15%. Twenty-six Florida children and adults, presenting for emergency care of coral snake bites, were then administered a dose of intravenous antivenom equal to that used in the large animal model. The primary endpoint was mortality, supported by secondary endpoints related to neurotoxicity to ensure that survival was not the result of intensive care alone. All survived ($p=.015$), and none required ventilatory support. One subject required a second antivenom dose; comparison of this subject's data with the animal model confirmed that he had received a particularly high venom load.

Overall, these studies established the effectiveness and appropriate dose of antivenom for treatment of neurotoxic envenomation by *M. fulvius*, without concurrent comparator treatment.

022: The neutralization of snake venom metalloproteases using a novel disintegrin antibody

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Snake venom metalloproteases (SVMPs) are a superfamily of zinc-dependent proteases making up 70% of total venom protein and are responsible for local systemic hemorrhaging in snake bite envenomations. They are classified into three different classes based on their functional domains designated as P-I (metalloproteinase), P-II (metalloproteinase/disintegrin) and P-III (metalloproteinase/disintegrin /cysteine-rich). In this study, the neutralizing ability of disintegrin polyclonal antibodies (anti-r-mojastin) from serum was realized on snake venom disintegrins, SVMPs, and crude venoms. Native and recombinant disintegrins were neutralized by abrogating their inhibitory activities on platelet function and cell migration. Using cation exchange High-Performance Liquid Chromatography (HPLC), an SVMP was isolated and confirmed by inhibition of metalloprotease activity using EDTA, detection of a disintegrin domain using anti-r-mojastin by immunoblotting and N-terminal sequencing. The SVMP had a minimal gelatinase dose (MGD) of 2.8 μg (4.4 μM) and was neutralized 100% *in vitro* by anti-r-mojastin. Indeed, three different crude *Crotalus atrox* venom's gelatinase activities were also neutralized by anti-r-mojastin. The minimal hemorrhagic dose (MHD) for crude *C. atrox* venom was 2.5 μg and was 32% neutralized by anti-r-mojastin. Our results indicate that using a novel antibody targeting disintegrins to neutralize metalloprotease activity can be effective both *in vitro* and *in vivo*, highlighting a new anti-venom therapeutic approach. Finally, our results further suggest alternative and innovative strategies are warranted to treat snakebite envenomations effectively.

023: Validation of computational models for tertiapin-blocked neuronal Kir3.2 channels.

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Virtual screening and structure-based computational modeling hold great promise for advancing mechanistic understanding of venom peptide function on target proteins. For venom

peptide redesign and repurposing for drug discovery, accurate determination of the molecular determinants mediating potency and selectivity are prerequisite. Current structure-based in silico models of tertiapin-blocked Kir3 channels have not been experimentally validated, and to that end we have tested key predicted contacts through combinatory point mutations of both tertiapin and the Kir3.2 channel. Kir3.2 channels were expressed in the *Xenopus* oocyte system as Kir3.1/3.2 heteromers to record their function electrophysiologically in response to TPN and TPN variant peptides synthesized by solid-phase peptide chemistry. Our structure-based molecular dynamic simulation models predict a key electrostatic contact between the Kir3.2 channel turret (E127) and the basic R7 residue in tertiapin. Mutations of either the turret (E127R) or tertiapin (R7E) significantly impacted channel block, increasing the IC50 >50-fold, consistent with a critical role of this contact in toxin binding. However, the reverse donor-acceptor combination TPN R7E effects on Kir3.2 E127R channels, failed to restore channel block, indicating a limit to the structure-based model predictions. We also tested the impact of two TPN lysines via alanine substitutions K17A and K21A, where K21A was predicted to plug the channel at the selectivity pore entry site via electrostatic contacts with the S1 tyrosine carbonyl groups. Both TPN variants effectively blocked Kir3.2 channels with moderate effects on the IC50 value, indicating a complex multi-contact interface of toxin peptide with the channel outer vestibule. These results highlight the importance of experimental validation with structural models of venom peptide-target interactions, where together through iteration, a comprehensive understanding of the molecular interactions can emerge that will enable rationale redesign of tertiapin as a scaffold for other Kir channel blockers. The details to our approach and findings will be presented.

024: Mechanism of Glutamate ReceptorBlockby Acylpolyamines

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In 1986, Eugene Grishinand coworkers publishedthe first structure of an acylpolyamine toxin, argiopin (also known as argiotoxin 636 or ArgTX-636), from the venom of the orb weaver spider Argiope lobata [1]. Since then,over a hundred of acylpolyamines have been identified in venoms of orb weavers, agelenids, and wasps. Owing to their ability to block both insect and mammalian ionotropic glutamate receptors (iGluRs) with high affinity,these toxins became indispensable as neuroscience research tools. However, the molecular bases of acylpolyamine-iGluR complex formation and channel block remained unknown. We used cryo-electron microscopy to determine structures of AMPA-subtype iGluR assembled ofGluA2subunits in complex with argiopin and two related synthetic compounds[2]. It appears that upon opening of the extracellular gate, argiopin enters the pore of the activated receptor and binds tightly in the internal cavity and selectivity filter in an extended conformation.The positively charged polyamine tail of the toxin transverses the negatively charged selectivity filter and reaches the cytoplasm, whereas its bulky acyl head becomes trapped inside the inner cavity stabilizing the toxin inside the channel pore. Our structure also provides clues to how argiopin and other acylpolyamines become trapped inside the channel when the receptor deactivates and the channel gate closes. Since glutamate represents the primary excitatory neurotransmitter in the central nervous system,iGluRsare implicated in various devastating neurological diseases. Our structures will stimulate drug discovery targeting pathologies that are associated with excitotoxicity, includingamyotrophic lateral sclerosis (Charcot's disease), epilepsy, Alzheimer's and Parkinson's diseases. The work was supported by the Molecular and Cell Biology Program of the Russian Academy of Sciences.

025: α-Conotoxin TxID and its Mutants Targeting α3β4 nAChR Subtype

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The α3β4 nicotinic acetylcholine receptor (nAChR) is widely distributed in peripheral nervous system and some regions of central nervous system, such as medial habenula, interpeduncular nucleus, and pineal gland. Selective α3β4 nAChR antagonists were proved to reduce nicotine self-administration and pain sensation. The α3β4 subtype also associates with small cell lung cancer, food overconsumption and obesity. Presently high potent and selective antagonists target α3β4 nAChR are in paucity. Especially all the available ligands lack good selectivity between α3β4 and the closely related α6β4 nAChR subtypes, which limits our understanding of the physiological roles of these important receptors. Therefore, there is an urgent need to discover new ligands potently to block α3β4 nAChR subtype, which could discriminate α3β4 versus α6/α3β4 nAChRs.

Here we characterized an α-conotoxin TxID and its various mutants targeting α3β4 nAChR subtype systematically. TxID consists of 15 amino acid residues with two disulfide bonds of Cys (I-III) and Cys (II-IV) connectivity and C-terminal amidation. Synthesized TxID was tested on nAChRs heterologously expressed in *Xenopus laevis* oocytes. It showed that TxID was the potent α3β4 nAChR antagonists blocking rat α3β4 nAChRs with an IC_{50} of 12.5 nM, which also blocked the closely related α6/α3β4 with an IC_{50} of 94 nM. To distinguish between these two close subtypes, positional-scanning mutagenesis of TxID was performed to identify critical residues that confer potency for α3β4 nAChRs. The activities of 15 analogues (the 1^{st} generation) and TxID were tested on both α3β4 and α6/α3β4 nAChRs. There was one mutant, [S9A]TxID showed 46-fold greater potency for α3β4 versus α6/α3β4 nAChRs, which showed significantly improved selectivity for α3β4 versus α6/α3β4 nAChRs. It suggested a vital role of residues at position 9 on the selectivity of TxID.

Then more TxID analogues of the 2nd generation (20 mutants) with a single amino acid substitution in [S9A]TxID, as well as methionine oxidation and substitution were designed and synthesized. Pharmacological activities of all the mutants were evaluated and obtained a mutant, which only inhibited α3β4 nAChR but had no obvious effect on other nAChR subtypes. It revealed the most selective on α3β4 nAChR. NMR analysis of TxID and its more selective mutants showed their specific surface properties. The results provide further molecular insights and a guide to develop new drugs to treat α3β4 nAChR related diseases.

026: Snake venom PLA_2 as a ligand and modulator of various protein targets (hCFTR, hFXa, nAChR): mechanism of action and therapeutic potential

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Ion channels constitute a diverse family of membrane proteins that regulate a variety of physiological processes including neuronal/neuro-muscular transmission and fluid transport. Dysfunction of ion channels can lead to disease states that require therapeutic agents acting as potential stabilizers or correctors of ion channel dysfunction. Ion channels therefore constitute privileged targets for natural or synthetic ligands with pharmacological potential. Several high affinity receptor binders are present in snake venoms.

We discovered that one of these natural ligands present in rattlesnake *Crotalus durissus terrificus*, the CB subunit of crotoxin[1], possesses therapeutic potential against cystic fibrosis, acting as a positive allosteric modulator on CFTR Cl^- channel current[2]. Its beneficial function consists of stabilizing and correcting the dysfunction of mutated dF508CFTR responsible for cystic fibrosis[2]. We also reported that CB interacts with neuronal targets such as the proton gated ion channel GLIC, a bacterial homolog of the pentameric ligand-gated ion channel family and with the acetylcholine binding protein (AChBP). CB is a negative allosteric modulator of GLIC, since it inhibits a proton-gated ion channel activity[3]. Interestingly, CB also possesses anticoagulant activity, inhibiting blood coagulation by direct binding to human anticoagulant factor FXa, independently of its enzymatic activity[4].

The aim of our study is to understand the mechanism of action of CB, a multifunctional protein which exhibits neurotoxic, anticoagulant and anti–cystic fibrosis properties and to identify amino acid residues involved in these interactions with various protein partners. Using biochemical and biophysical methods (SPR, HDX-MS, molecular docking and X-ray crystallography) we investigated the binding interfaces between CB and three different protein partners: human CFTR, human FXa and nAChR. Since various CB targets regulate functions essential to life processes, the determination of PLA_2-receptor binding sites represents a challenging objective in receptor-channel biochemistry and pharmacology.

This study provides novel perspectives for the development of new drugs against cystic fibrosis and non-competitive FXa inhibitors with anticoagulant properties, and the identification of new allosteric modulators of the nAChR family.

027: Effects of MLO crude venom, PLA2 and metalloproteinases enzymes on cardiac cells.

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Macrovipera lebetina obtusa (MLO) snakes presented by several sub-species, which are characterized by a very specific combination of components. Along with common enzymes characteristic of Vipera species, *MLO* venom contains unique components specific for "obtusa" sub-species, such as disintegrin obtustatin. *MLO* venom contents metalloproteinases, phospholipase A2, serine proteases, L-alfa amino acid oxidase, few kinds of disintegrins and some other active agents. The cluster of *MLO* venom components, such as metalloproteinases, obtustatin, C-type lectins and few others are known as cell adhesion inhibition molecules, which are breaking up integrins and cadherins or bind to them. Therefore, the adhesion affecting properties of *MLO* venom and its action on cell binding in tissue culture is of great interest. We studied **effect of venom of *MLO* living in Armenia on adhesion and metabolic activity of myocardial cells [cardiomyocytes (CM) and cardiac fibroblasts (CF)] and identification of major effector components of MLO venom.**

Attachment properties of both CM and CF were affected by MLO venom in a dose-dependent manner. Time exposure also has an effect on CMs and CFs attachment to the substrate and to cell-to-cell contact. Longer exposures times result in higher detachment even at the same MLO venom concentrations. Interestingly, both cell types demonstrated increase in metabolic activity upon exposure to non-lethal doses of crude venom.

Analysis of individual components of MLO venom resulted improved adhesion of both CM and CF upon inhibition of metalloproteinases by EDTA-Na_2 chelating agent or complete block of PLA2 enzyme by bromophenacyl bromide. These results indicate that the aggressive detaching effect of *MLO* venom is delivered not by an individual component of the venom, but rather is a combinatorial effect of several active ingredients.

028: Sea anemone peptides: therapeutic leads, pharmacological tools and new folds

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Sea anemones are a rich source of peptides that are potent and often selective probes of the structure and function of ion channels, and are of significant interest to the pharmaceutical and biotech industries as both therapeutic leads and pharmacological tools.[1] I will describe our studies on peptides from sea anemones[2,3] that are potent blockers of the voltage-gated potassium channel Kv1.3. Kv1.3 channels play a major role in the activation of effector memory T cells, which are involved in autoimmune diseases such as multiple sclerosis, psoriasis, type 1 diabetes and rheumatoid arthritis. Peptide blockers of this channel selectively inhibit the activation of T_{EM} cells and show considerable potential as therapeutics for autoimmune diseases.[2,4]

Given the promise of sea anemone peptides, we have continued to explore additional species for new peptides. The ShK fold is clearly widespread in nature, not only in the phylum Cnidaria, but also in parasitic worms[5,6] mammals[6] and even plants. A comparison of available ShK sequences based on various physicochemical properties reveals that they cluster into discrete sub-sets; the relationships among these clusters, their functional activity and potential as therapeutics are currently being explored, but it is clear that this fold can support other activities beyond potassium channel blockade. Transcriptomic and proteomic studies of sea anemones have also identified many novel peptides, the distribution of some of which has been mapped using imaging mass spectrometry.[7] The structure and function of some of these novel peptides will be described.

029: Development of an “app” to assist management of mushroom poisoning

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Mushroom poisoning is a significant medical issue within clinical toxinology, particularly in some regions where it may cause more hospital presentations and potentially fatal cases than envenoming causes such as snakebite. We have recently revised the classification of the substantial number of syndromes of mushroom poisoning. Provision of clinical advice to colleagues managing cases of mushroom ingestion and poisoning requires rapid access to a diverse set of data. To assist this one of us (JW) has developed a database-driven application ("app") for mushroom poisoning running on iOS. We sourced data on the classification of mushroom poisoning, clinical features and treatment of mushroom poisoning, associated diagnostic algorithms and taxonomic identification from files previously developed within our department. Key information was classified into heading types to provide a framework, allowing the development of an "app" using FileMakerPro database software, written to run on iOS on an iPadPro. This master version was populated with the existing data, sourced as above, to produce a fully functioning "app".

The "app" uses a series of layouts to provide ready access to information. Layout 1 provides information on each of the current 21 groups and subgroups of mushroom poisoning, accessed through a series of 7 "tabs" (overview; mushrooms; toxins; clinical; treatment; diagnostic; literature), each providing immediate access to fields relevant to the "tab" heading, with scrolling through fields with large amounts of text. Dedicated function "buttons" at the top of the page allow immediate access to other sections of the "app"; these include the diagnostic algorithm, mushroom species records and mushroom poisoning literature. The diagnostic algorithm section allows easy progression through the algorithm, designed to assist in determining the most likely type of mushroom poisoning based on clinical features, for cases where the identity of the consumed mushrooms is uncertain. The mushroom species records can contain details of individual mushroom species, including diagrammatic keys to identification. The literature section allows incorporation of publishing details for papers, complete abstracts and notes on the publication and can also hold pdf copies of the entire publication, if desired. A separate section provides a system for recording mushrooms involved in cases of poisoning, with diagrammatic assistance in logging key taxonomic features, which can then be used to compare with existing species records. The iPadPro can take photos of mushrooms being logged and these are uploaded into the relevant record.

This "app" has been developed to assist our Toxinology Dept. service provision when consulted on cases of mushroom poisoning, providing rapid and easy access to key information. Rapid identification of mushrooms involved in poisoning, or the type of poisoning presenting, can be instrumental in optimising medical management. Early intervention can lessen or avoid the

potentially serious complications of some forms of mushroom poisoning. The “app” will continue to be developed and progressively populated with mushroom identification data and relevant literature, with a view to making it available widely in the future.

REGULAR ORAL COMMUNICATIONS

O-030: Pore-forming toxins from sea anemone *Heteractis crispa*: Diversity and pharmacological potential

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Pore-forming toxins of sea anemones named actinoporins are interesting as tools for studying protein-membrane interactions, as well as being potential therapeutic agents for cancer therapy.Certain attention is now directed to the investigation of the actinoporin action on target organs and different cell cultures, as well as to the creation of actinoporin immunoconjugates with different ligands for selective killing of parasite and tumor cells. We demonstrated that actinoporin from *H. crispa* exhibited an antitumor effect and suppressed IGF-induced tumor transformation of JB6P + Cl41 mouse epithelial cells. This effect was found to be due to the induction of p53-independent apoptosis and the inhibition of the activity of the oncogenic nuclear factors AP-1 and NF-κB.

We have performed a comprehensive investigation of *H. crispa* actinoporins, including molecular cloning, modeling, and biological activity testing. The approach allowed us to establish a variety of non-abundant transcripts encoding the sea anemone venom actinoporins. This diversity is determined by the existence of the actinoporin multigene family of 47 representatives, at least. The phylogenetic analysis and molecular modeling data demonstrate that the combinatorial library of actinoporins is represented by three groups differing from one another by the structural features, as

well as by the magnitude and direction of the N-terminal dipole moment. The functional analysis of some recombinant actinoporins revealed that *H. crispa* actinoporins grouping was consistent with the different hemolytic activity of their members. We strongly assume that the direction of the N-terminal dipole moment tightly reflects the actinoporins' power to possess hemolytic activity.The cytotoxic activity of recombinant Hct-A2 on different tumor cells was tested.

O-031: Whole Genome Sequencing of a Japanese Endemic Pit Viper, Habu, *Protobothrops flavoviridis* Reveals Accelerated Evolution of Venom Protein Genes Enriched in Microchromosomal Regions.

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Evolution of novel traits is a challenging subject in biological research. Several snake lineages developed elaborate venom systems to deliver complex protein mixtures for prey capture. To understand mechanisms involved in snake venom evolution, we decoded here the ~1.4-Gb genome of a Japanese endemic pit viper, habu, Protobothrops flavoviridis. We identified 60 snake venom protein genes (SV) and 246 non-venom paralogs (NV), belonging to 18 gene families. Molecular phylogeny revealed an early divergence of SV and NV gene copies, suggesting that one of the four copies generated through two rounds of whole-genome duplication was modified for use as a toxin in the venom. Among them, both SV and NV gene families of the four major venomic components, metalloproteinase, serine protease, C-type lectin-like protein and phospholipase A2 were extensively duplicated after their diversification. An accelerated evolution was evident in their SV genes but not in NV counterparts. On the other hand, genes for the other 14 families those were not extensively duplicated showed no evidence of accelerated evolution. We also observed that venom-related SV and NV gene copies are significantly enriched in microchromosomes than in macrochromosomes, suggesting the implementation of the genomic architecture in the multiplication and the accelerated evolution in the venom-related genes.

O-032: Snakebite and antivenom management in Nepal

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Snakebite in Nepal is an important occupational injury affecting farmers, plantation workers, herders and fishermen. The majority of snakebites occur in the southern alluvial plains of the terai region with hot tropical climate and high human population density. Most bites (68%) occur during the rainy season, from May to October, which corresponds to the peak period for agricultural work. The victims' mean age is 32 years, and the majority is male (60%) and literate (69%). Open-style housing and the practice of sleeping on the floor also expose people to bites from nocturnal snakes.

Many victims in rural areas die at home unrecorded and properly designed population- based studies suggest that hospital figures greatly underestimate the burden of morbidity and mortality.

We analyzed and presenting the data from 2000-2010 obtained by Epidemiology Disease Control Division, Department of Health Services. Comparison of venom composition of India snake venom used in anti venom production with corresponding Nepalese snake is essential. ED50 (effective neutralizing dose of antivenom) of India antivenom against medically relevant snakes of Nepal is crucial to decide on the inclusion of venoms from Bungarusniger,B. walli, B. lividus, Najakaouthia, Trimeresurophisalbolabris and Ovophismonticola in the antivenom production for Nepal. Until more effective antivenom is obtainable, the available antivenom should be provided to qualified health facilities in affected areas. Because of possible Indian antivenom incompatibilities against B. lividus, B. niger and N. kaouthia bites and poor health facilities in Nepal, adopting the best preventive measures of snake bite would be desirable and economic. Therefore, education tools should be developed and regular trainings should be organized.

Snakebite has been declared a global public health emergency. Treatment with specific antivenom is considered the only cure. In Nepal, estimated 40,000 vials of antivenom are needed each year to control the effects of snakebite envenoming. In Nepal, government does allocate sufficient funds for keeping enough antivenom. To improve national availability, larger laboratories require clearer description of market size, in order to plan long-term manufacturing strategies.

O-033: Understanding the Local Tissue Necrosis of the Bitten Victim from Cobra Snakebite

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Victims suffered from cobra snakebite usually exhibit clinical symptoms with systemic neurological abnormality such as muscle weakness/respiratory arrest and/or local tissue damage such as toe gangrene/extensive necrotizing fasciitis. The toxins responsible for the neurological symptoms are three-fingered neurotoxins (NTXs). But, the mechanism responsible for the severe necrotizing tissues is not clear. In this presentation, we compare the proteomic and biochemical properties of venoms two from cobra species *N. atra* from Taiwan and *N. Nivea* from south

Africa. We also determined three-dimensional structures of high molecular weight toxins such as PDE, 5NT, and LAAO from *N. atra* for the first time in order to understand their biological targets. The results suggest that the actions of three-fingered cardiotoxins (CTXs, or named as cytotoxins) and biochemical activities of PDE, 5NT, LAAO and PLA2 synergistically causing severe tissue necrosis. Three-dimensional structures of the PDE, 5NT and LAAO from *N. atra* are found to be the structurally homologous to human immune system factors ENPP1, CD73 and iL4I1, respectively. These results reveal an important role of the micro-environmental change in the bitten area. Both cytotoxic CTXs and metabolic toxins from cobra venom significantly perturb the metabolites of the damaged tissues and severely prevent wound healing process of the bitten victims.

O-034: Rational design and development of anti-venom drugs for snakebites based on the endogenous inhibitors from Japanese Viper

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Venomous snakes have endogenous serum proteins that neutralize the toxicity of their toxins. We previously identified new class of endogenous inhibitors from Japanese vipers (*Protobothrops flavoviridis* and *Gloydius blomhoffii*) sera. They are named small serum proteins (SSP-1 to -5), belong to the prostate secretory protein of 94 amino acids (PSP94) superfamily. Interestingly, these inhibitors target different toxins from snake venoms. So far, the details of molecular interactions between these endogenous anti-toxin proteins and toxins and the mechanism of inhibition are unclear.

Here we describe the first crystal structure of SSP-2 with CRISP family toxin with ion-channel blocking activity and define the structural basis of SSP-meditated inhibition of toxin

activity. Our discussion focuses on molecular interaction between them and we propose the mechanism of abrogation of Ca2+ channel inhibitory activity of the CRISP toxin based on the crystal structure. These molecular interactions of an endogenous inhibitor with the toxin explain the physiological role of SSPs in the natural resistance towards divergent toxins. We have investigated of potential utility of SSPs as therapeutic drug for snakebites. Using SSP- immobilized affinity column chromatography, we have identified target toxin from crude venoms of several snakes (*Gloydiushalys brevicaudus, Bothrops jararaca, Bothrops medusa, Naja naja, Naja kaouthia*, and *Oxyuranus scutellatus*, and *Notechis scutatus*). We have used Protein-Protein interaction analyses and inhibitory activity for evaluation of affinity and specificity on toxin. The molecular mechanism of natural resistance in snakes may help in understanding the specificity and selectivity of these endogenous inhibitors and in designing better therapeutic agents for the treatment of snakebite victims.

O-035: Involvement of Necroptosis and Ferroptosis pathway signaling in *Hemiscorpius lepturus* venom -induced acute kidney injury

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Hemiscorpius lepturus venom, as a nephrotoxic agent, is related to hemoglobinuria and proteinuria as the main important clinical signs in acute kidney injury (AKI). The sever envenomation in patients associated by hemolysis and hemoglobinuria. Cell death mode in AKI pathophysiology is related to necroptosis, ferroptosis and inflammatory cytokine expression. Unfortunately, the molecular and cellular mechanism of hemolysis induced AKI by *Hemiscorpius lepturus* is not clear.

Male albino mice were with an SC injection of venom (1, 2.5, 5 and 10 mg/kg), while sham group received vehicle only. After 1 and 7 day, the animals were assessed for urinalysis and renal markers. Then, the animals were sacrificed and kidney was collected to analysis for assessment of cellular and molecular analysis. On the other hand, stereological techniques in kidney were used to estimate nephron volume and number.

Our data revealed up-regulation of TNF-α, TLR-4, RIPK3, and MLKL gene expression in RT-PCR experiments is related to renal NGAL over expression. Moreover, the MDA level was increased in venom treated mice in dose-dependent manner similar to ACSL-4 over expression. It seems that envenomation-induced necroptosis may be contributed to hemoglobinuria mediated AKI. Besides, Dis-regulation of lipid metabolism revealed involvement of ferroptosis in hemoglobinuria induced AKI.

Overall, it supposed that venom-induced AKI is related to co-existence of two separate pathways of regulated necrosis and inflammatory environment in hemoglobinuria case after venom treatment needs combination therapy.

O-036: Purification and partial characterization of AIP1: a novel protein from Sea-Star (*Astropecten indicus*) coelomic fluid

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Echinoderms especially sea-stars or starfish are known for their immense regenerative potential. The coelomic fluid of echinoderms is a reservoir of immunocytes, coelomocytes, cytokines and other chemicals which are responsible for the regenerative potential and other bio-activities. Despite its vast potential, no wound healing agent has been purified from them. In this study, the coelomic fluid of Sea star *Astropecten indicus* was fractionated using ion exchange chromatography and size exclusion HPLC to yield a potent protein AIP1. The purified protein showed fibrinogenolytic and fibrinolytic activity at dose of 10µg. It promoted ADP and collageninduced platelet aggregation. Proteolytic activity of AIP1 was inhibited post treatment with EDTA, indicating its metallo-proteinase nature. The protein AIP1 showed no cytotoxicity on A549, HaCaT or HEK293 cells *in vitro.* It was also devoid of hemolytic and phospholipase activities. Scratch wounds created on surface of A549, HaCaT and HEK293 cells showed fasterwound closure

rates post treatment with AIP1. Experimental data was substantiated with peptidemass fingerprinting analysis of AIP1 peptides which yielded one domain homologous to von Willebrand factor superfamily-A domain using UniProt KB. AIP1 is the first thrombolytic and wound healing protein reported from the sea-star *Astropecten indicus*.

O-037: Expression of vascular endothelial growth factor in S-180 sarcoma-bearing mice after treatment with obtustatin and Macrovipera lebetina obtusa snake venom

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Macrovipera lebetina obtusa (MLO) is a snake whose venom consists of 38 proteins that belong to only a few major protein families, including enzymes and proteins without enzymatic activity. Among them is the shortest known monomeric disintegrinobtustatin. Recently, we have shown MLO venom and obtustatin exhibited a potent therapeutic effect on S-180 sarcoma melanoma progression assumedly due to the inhibition of angiogenesis. Since vascular endothelial growth factor (VEGF) plays an essential role in angiogenesis in the present study we investigated the effect of MLO snake venom and obtustatin on VEGF expression in S-180 sarcoma bearing mice model. The VEGF expression was increased after treatment with obtustatin and MLO venom, which was more significant in case of MLO venom. Such a phenomenon can be explained by the ability of the obtustatin and MLO venom to inhibit angiogenesis which in turn can cause hypoxia in tumor tissue. Under hypoxic conditions hypoxia-inducible factor1-α (HIF-1α) is activated enhancing HIF-1α-dependent transcriptional activation of VEGF. This hypothesis will be justified in future by checking the HIF-1α expression in the control and treated groups.

O-038: Development of an antivenom for Vipera and Macrovipera bites of western and eastern Europe

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Production of equine $F(ab')_2$ antivenom against western European *Vipera* species has been discontinued by Sanofi Pasteur, and there is a pressing need for a replacement antivenom. We developed a product with expanded coverage that includes viperid snakes of both eastern and western Europe.

Venoms were obtained from Latoxan (France), and the immunizing combination represented *Vipera aspis* (12%), *V. ammodytes* (10%), *V. berus* (34%), *V. (Montivipera) xanthina* (16%), *Macrovipera lebetina obtusa* (10%), *M. l. cernovi* (6%), *M. l. turanica* (6%) and *M. l. schweizeri* (6%). Healthy, 5 to 7 year old, castrated-male horses were immunized (with alternating incomplete Freund´s and Alum adjuvants) using venom doses from 150 mcg to 15 mg, increasing over the course of six months to achieve sufficient titers for hyperimmune plasma production. Thereafter, blood was collected every four weeks, alternating with a reinforcement immunization of 15 mg of the venom mixture, 10 days prior to bleeding. Blood collection provided an average of 3.0 L of plasma; and the corresponding red cell pack was returned to the horse, using a peristaltic pump, within 3 hours.

Highly refined (> 95% $F(ab')_2$) lyophilized antivenom was then manufactured using a closed system in which the hyperimmune plasmas were pepsin-digested, precipitated with ammonium sulfate, ultrafiltered to remove digested peptides and the excess of precipitating salt, nanofiltered to remove potentially present equine viruses and formulated with glycine and sucrose for filling and lyophilization.

Each vial of the final product, using the mouse test, neutralizes a minimum of 4.8 mg of *V. aspis*, *V. ammodytes*, *V. berus* and *V. (Montivipera) xanthina* venoms, and a minimum of 6.8 mg of venom of any of the *M. lebetina* subspecies.

O-039: Equine $F(ab')_2$-based antivenom preparation by simultaneous caprylic acid fractionation and pepsin digestion

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Antivenoms obtained from hyperimmune animal plasma have been the only specific and effective therapy against snakebites. Design of the ideal process preceding their commercial scale production should be guided by the tendency to refine IgGs or their antigen-binding fragments from residual plasma proteins in the most straightforward way, providing final product of retained potency and reduced side-effects inducing potential.

Here we report simple, feasible and economically viable downstream processing strategy for Vipera ammodytes-specific antivenom preparation that has been guided by the imperative of constant maintenance of $F(ab')_2$ fragments in solution, as a precautionary measure against their conformational or structural change due to precipitation or binding to chromatographic support. Thus, protection of the active principle from possible degradation and/or aggregation was ensured. Also, for the first time caprylic acid fractionation of horse plasma was successfully co-performed with the pepsin digestion step. Developed mode employing simultaneous precipitation of contaminating proteins together with Fc fragment removal from IgGs resulted in $F(ab')_2$-based product that was additionally polished by anion-exchange chromatography in the flow-through mode, under conditions that prefer binding of processing by-product traces, especially pepsin. Such compressed manufacturing procedure yielded over 70% of active principle, as properly and reliably estimated by several in vitro methods. The final preparation was distinguished by high purity and preserved in vivo neutralisation efficacy in comparison to the starting plasma pool.

O-040: The acute effects of snake venom CRiSP toxins on blood and lymphatic endothelial cell permeability: new insights into the pathophysiology of snakebite

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Snakebite is a huge global health problem, causing serious injury to 2.7 million men, women and children and claiming an estimated 125,000 lives annually. In spite of its huge toll on human health, very little is known of the pathophysiology of snakebite. Although several studies have reported the lymphatic system plays a critical role in venom absorption and distribution following snakebite, the role of the lymphatic system in envenomation remains unclear. Several snake venom Cysteine-Rich Secretory Proteins (svCRiSPs) have been shown to possess ion channels-blocking activities and affect the activity of vascular endothelial cells.

It is our hypothesis, based on the limited information available in the literature and the preliminary results we have obtained with Hellerin, a newly discovered rattlesnake CRiSP, that svCRiSPs play an important role in the pathophysiology of snakebite by initiating a proinflammatory response in tissues at the site of the snakebite and dramatically increasing vascular permeability. The aim of this work was to investigate the role of svCRiSPs from five of the most medically significant species of North American vipers (Rattlesnakes: *Crotalus atrox*, *C. adamanteus*, *C. horridus*, *C. scutulatus scutulatus*; Cottonmouths: *Agkistrodon piscivorus piscivorus*) in snakebite, focusing specifically on the effects of these toxins on the function of the blood and lymphatic vessels located in proximity to the bite. Knowledge gained from these studies will provide insights into the molecular mechanisms that underlie the effects of svCRiSPs on vascular function and will contribute to a new level of understanding of the pathophysiology of snakebite and the development of novel therapeutic strategies for the treatment of snakebite and possibly other vascular and lymphatic diseases.

O-041: Novel applications for snake venom disintegrins

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The long-term mission of the National Natural Toxins Research Center (NNTRC) is to characterize novel snake venom toxins that have biomedical applications and to develop innovative new therapies for snakebites. We have selected novel toxins that have anticancer properties and can

be used to create screening / neutralizing antibodies. Therefore, we have focused our attention on the snake venom disintegrins. Disintegrins are low abundant peptides found in many North / South American Crotalid and Old-World vipers. Though these toxins are ubiquitously expressed in many species, very little is understood about the molecular basis for their toxicity and potential for the inhibition of cancer cell growth. Herein, we highlight the current work at the NNTRC with snake venom disintegrins. Our results suggest that antibodies produced against these toxins present novel neutralizing ability for gelatinase and hemorrhagic activities against crude venom. The isolated toxins can inhibit melanoma cell migration by modulation of various transcription factors resulting in apoptosis. We have functionalized disintegrins which can be used as molecular probes to visualize endothelial and cancer cells. Proteomic analysis of disintegrins interactions reveal pathways and networks which shed new light on downstream molecules in cells that are being affected providing new details for the elucidation of the disintegrins mechanism of action**.** Understanding this will lead to design and synthesis of drugs / peptides using disintegrins as a template to enhance novel cancer therapeutics or designing probes for cellular imaging. Indeed, using antibodies to target the disintegrin domain of snake venom metalloproteases and neutralizing their activity presents a new paradigm for treating snake bites targeting a single molecule. Taken together, disintegrins present enormous potential for the diagnosis, treatment and evaluation of cancer and for the development of a novel antibodies to treat snakebites.

O-042: Interaction of gating modifier toxin Hm-3 with voltage-sensing domains of Nav1.4 sodium channel: structural view on the membrane-mediated binding

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Voltage-gated Na^+ (Na_V) channels contain domains that have discrete functionalities. The central pore domain allows current flow and provides ion selectivity, whereas peripherally located four voltage-sensing domains (VSD-I/IV) are needed for voltage-dependent gating. Certain

mutations trigger a leak (gating-pore) current through VSDs leading to various diseases. For example, hypokalemic periodic paralysis (HypoPP) type 2 is caused by mutations in the S4 voltage-sensing segments of VSDs in the skeletal muscle channel $Na_V1.4$. The gating modifier toxin Hm-3 (crab spider *Heriaeus melloteei*) inhibits leak currents through such mutant channels and represents useful hit for HypoPP therapy.

To investigate molecular basis of Hm-3 interaction with $Na_V1.4$ channel, we studied isolated VSD-I and VSD-II by NMR spectroscopy in membrane mimicking environment provided by detergent micelles. Hm-3 partitions into micelles through a hydrophobic cluster formed by aromatic residues and interacts with both VSD-I and VSD-II by the prolonged beta-hairpin. The toxin demonstrates higher affinity to VSD-I. The model of the Hm-3/VSD-I complex was built using protein-protein docking guided by NMR restrains. Hm-3 interacts with the S3b helix and the S3–S4 extracellular loop of VSD-I through electrostatic and hydrophobic interactions.

The topology of the Hm-3/micelle interaction is not significantly altered upon toxin binding to VSD-I. A major free energy contribution to the stability of Hm-3/VSD-I complex comes from the initial partition of the toxin into the micelle. This suggests membrane-mediated mechanism of Hm-3/$Na_V1.4$ interaction. The toxin initially anchors onto the membrane surface and then forms the complex with the S3b-S4 loop; thus blocking movement of the voltage-sensor helix S4. The Hm-3 binding probably induces some allosteric changes in S4 helix that prevents development of gating-pore currents through VSD-I.

Work was funded by the Russian Science Foundation (#16-14-10338).

O-043: Cholinergic ligands from different sources as research tools and potential drugs

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To date, a huge number of compounds from different sources interacting with nicotinic acetylcholine receptors (nAChRs) have been isolated. The structural variety of such cholinergic ligands is a good basis for successful search and targeted design of compounds highly effective and selective towards distinct nAChR subtypes. Taking into account the involvement of distinct receptor subtypes in a number of diseases and pathologies (myasthenia gravis, Alzheimer and Parkinson diseases, autism, different types of pain, nicotine addiction), new cholinergic ligands may be useful for the diagnostics of these diseases or even the development of appropriate drugs, as well as they may also serve as instruments for the study of nAChRs. For example, we have identified indole compounds from the toad *Bufo bufo* and nudibranch mollusk *Hermissenda crassicornis*, interacting with the α7 nAChR subtype. More complex in structure new plant bisbenzylisoquinoline alkaloids, which have recently been isolated from a Matis tribe arrow poison, showed higher selectivity towards muscle-type receptors. Through the application of useful tools for fundamental studies of different nAChR subtypes we are trying to develop also promising substances for clinical use. Design of effective markers of peptide nature for α7 nAChR, involved in Alzheimer and mild cognitive diseases, on the basis of α-conotoxin PnIA[A9R] was successfully carried out by using computer protein surface topography method. Practical application has been achieved in the development of a peptide blocker of muscle-type nAChR - azemiopsin from viper *Azemiops feae* venom, as a peripheral myorelaxant.

This work was supported by the grant #18-04-01366 from Russian Foundation for Basic Research.

O-044: Detoxified Tetanus Toxin (TETIM) – A superb nano-carrier for retro-axonal gene delivery to motor neurons in bypass of blood brain barriers

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Trans-vascular barriers impose immense challenges to the delivery of beneficial cargo and genes to the central nervous system. Tetanus toxin (TeTx) binding fragment (Hc) has shown promises as a neuron targeting vehicle in neuronal cultures and animal models, but failed to facilitate long-range delivery of reporter or therapeutic vectors. As it emerges, H_C has the capability to target neurons, whereas the entire molecule is necessary for long-range trafficking that ensures central delivery. We investigated motor neuron targeting and gene delivery potentials of a protease-inactive TeTx mutant (TeTIM) fused to core streptavidin (CS) in comparison with TeTx binding fragments. In neuron binding and internalization assays, TeTIM was superior over TeTx fragments in cultured neurons. It also displayed faster clearance from the motor nerve terminals after peripheral injection with greater ability for retrograde propagation to the spinal cord and brain stem neurons. Importantly, unlike TeTx binding fragments retrogradely labeling only choline acetyltransferase (ChAT) positive neurons, CS-TeTIM was detected in both ChAT- positive and -negative neurons, the latter being putative interneurons. In experiments with targeting and delivery of the reporter green fluorescence protein (GFP) and therapeutic SNAP-25 resistant to botulinum neurotoxin type A, E and C1, CS-TeTIM also out-performed TeTx fragments, enabling motor neurons to express GFP and become resilient to challenge by type A and E toxin in vivo, whereas the same virus conjugated to CS-HC proved ineffective. Finally, we demonstrate the utility of CS-TeTIM for gene delivery to spinal cord motor neurons of amyotrophic lateral sclerosis (ALS) mouse model SOD1 (G93A). These findings show that as a nano-carrier, full-length inactive TeTx significantly exceeds H_C and HC of TeTx and exploits more efficiently the retrograde transport and trans-synaptic transfer mechanisms for delivery. Such qualities make TeTIM a more suitable research probe and neuron-targeting vehicle for delivery of viral vectors to central neurons.

O-045: Sea anemone *Heteractis crispa* produces a pool of peptides active on ASIC channels

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Acid sensing ion channels (ASICs) expressed in a central (especially ASIC1a subtype) and in a peripheral nervous system (especially ASIC3 subtype) can instantly responded to extracellular acidification and be involved in various physiological and pathological processes. Until recently sea anemone venoms have been considered as a source of toxins modulated activity of homomeric ASIC3 or ASIC3-containing heteromeric channels. Today we present the structure-function investigation of APETx-like peptides from the sea anemone Heteractis crispa possessed modulating activity to both ASIC1a and ASIC3 channels. By the combination of chromatographic technique, Edman degradation and tandem mass spectrometry three peptides named π-AnmTX Hcr 1b-2, -3, -4 (4500–4700 Da) were obtained and structurally characterized. In electrophysiological experiments on oocytes the peptides inhibited ASIC1a currents at micromolar concentrations onto 64–86%. In addition, π-AnmTX Hcr 1b-2 and -3 almost completely inhibited ASIC3 current but at saturating concentrations, while π-AnmTX Hcr 1b-4 provided the significant (up to 207%) potentiating of the transient current through ASIC3 channels. Using RACE techniques, we determined five other APETx-like peptide isoforms in the venom that allowed us to assume the existence of the combinatorial library of these structural class peptides in sea anemones. The modeling approach revealed a difference of surface electrostatic potential distribution among the H. crispa peptides as well as the structural template, APETx2 (PDB ID: 1WXN). Major peptide, π-AnmTX Hcr 1b-2, possessed in vivo antihyperalgesic activity in a model of acid-induced muscle pain. The study of molecular diversity and peptides modeling was funded by RFBR, according to the research project № 18-38-00387; the electrophysiological study on heterologously expressed ASICs was supported by Russian Science Foundation Grant 18-14-00138.

O-046: Toxicity and microglia activity in murine induced by *Macrovipera lebetina obtusa* venom with inhibited enzymatic activity

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The toxicity and microglia activity of rat brain following exposure of the *Macrovipera lebetina obtusa* (MLO) venom with inhibited enzymatic activity was investigated.

Toxicological data was calculated using Behrens and Müller-Tainter methods. Ca^{2+} ATPase method of rat brain microcirculatory bed and microglia staining was used. Phospholipase A_2 ($svPLA_2$) enzymatic activity was inhibited by bromphenacyl bromide, metalloproteinases (svMPs) activity – by EDTA- Na_2. Surface, size of brain microglial cells and staining intensity were quantified using ImageJ software.

The toxicity of MLO crude venom (LD_{50}, intraperitoneal injection, IP) in rats was 1.86 mg/kg and in mice – 1.74 mg/kg. Toxicity of MLO venom with inhibited $svPLA_2$ in mice was 3.39 mg/kg and with inhibited metalloproteinases - 6.84 mg/kg. The vasodestructive action of inhibited metalloproteinases was decreased and resulted in less changes in ATPase activity compared to the intact MLO venom effect. Decreased activity of microglial cells of different regions of venom affected brain compared to intact venom effect was also demonstrated.

Although $svPLA_2$ contents in MLO venom is about 14.6%, it has a significant tribute in venom toxicity. $svPLA_2$ inhibition did not influence drastically on the hemorrhagic action of MLO venom, but it was noted some changes in hemorrhagic loci picturing in microphotographs. The degree of activation of microglia and changes of its form, size, and position were well correlated with the enzymatic activity of svMPs.

POSTER ABSTRACTS

P-001: Mechanisms underpinning the permanent muscle damage induced by snake venom metalloprotease

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Snakebite is a major neglected tropical health issue that affects over 5 million people worldwide resulting in around 1.8 million envenomations and 100,000 deaths each year. Snakebite envenomation also causes innumerable morbidities specifically loss of limbs as a result of excessive tissue/muscle damage. Snake venom metalloproteases (SVMPs) are a predominant component of viper venoms, and are involved in the degradation of basement membrane proteins (particularly collagen) surrounding the tissues around the bite site. Although their collagenolytic properties have been established, the molecular mechanisms through which SVMPs induce permanent muscle damage are poorly understood. Here, we demonstrate the purification and characterisation of an SVMP from a viper (Crotalus atrox) venom. Mass spectrometry analysis confirmed this protein as a group III metalloprotease and therefore this has been referred as CAMP (Crotalus atrox metalloprotease). CAMP displays both collagenolytic and fibrinogenolytic activities and inhibits platelet aggregation. To determine its effects on muscle damage, CAMP was administered into the tibialis anterior muscle of mice and its actions were compared with cardiotoxin I (a three-finger toxin) from elapid (Naja pallida) venom. Extensive immunohistochemistry analyses confirmed that CAMP significantly damages skeletal muscles by attacking the collagen scaffold and other important basement membrane proteins, and prevents their regeneration through disturbing the functions of satellite cells. In contrast, cardiotoxin I destroys skeletal muscle by damaging the plasma membrane, but does not impact regeneration due to its inability to affect the extracellular

matrix. Overall, this study provides novel insights into the mechanisms through which SVMPs induce permanent muscle damage.

P-002: Study on the mechanism of antibacterial action of the peptides p-BthTX-I and its Disulfide-Linked Dimer $(p\text{-BthTX-I})_2$

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Based on the antimicrobial activity of the bothropstoxin-I (BthTX-I) and on the premise that the C-terminal peptide of Lys49 myotoxin can reproduce the microbial activity of the parent protein, our research group synthesized and characterized a peptide derived from the C-terminal region of BthTX-I (p-BthTX-I, sequence: KKYRYHLKPFCKK) and its disulfide-linked dimer $(p\text{-BthTX-I})_2$. In a previous work, p-BthTX-I and $(p\text{-BthTX-I})_2$ showed antimicrobial activity against both gram-negative and gram-positive bacteria, including multidrug-resistant bacteria. Moreover, p-BthTX-I and $(p\text{-BthTX-I})_2$ did not promote lysis or form membrane pores and did not presented activity against *C. albicans*, erythrocytes, epithelial cells, or macrophages, showing a possible specificity against prokaryotic cells. Then, the present work aimed the characterization of peptides mechanism of action. Inhibitory activities of DNA gyrase and Topoisomerase IV showed that p-BthTX-I and $(p\text{-BthTX-I})_2$ were able to inhibit both enzymes in the concentration of 200 μM. The fluorescence quenching technique using trypan blue by *flow cytometry showed that the carboxyfluorescein labelled-peptide* [$(p\text{-BthTX-I})_2$-CF] *when incubated with E.* coli, has an improved cell penetration compared to the *S. aureus*. Studies on mechanism of action of cell death *induced by the peptide* $(p\text{-BthTX-I})_2$ showed loss of membrane integrity in *E. coli* and *S. aureus*; however for *E. coli*, the peptide presented a mechanism of cell death apparently different from that presented by *S. aureus*, characterized by the presence of necrosis and late apoptosis. Scanning electron microscopy in *E. coli*, and *S. aureus*, showed morphological changes in the cells, with superficial deformity,

appearance of wrinkles, bubbles and formation of vesicles, suggesting a mechanism of action in the cellular membrane. Our results demonstrate that peptides analysed are promising prototypes for new strategies to treat infections caused by multidrug-resistant bacteria.

P-003: New Kunitz-peptide of *Heteractis crispa* with a propeptide in the precursor structure interacts with serine proteases and exhibit neuroprotective activity

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Sea anemone venoms are rich in peptide toxins, distinguished traditionally on four groups: pore-forming toxins, sodium and potassium channel toxins, and Kunitz-peptides. As known sea anemone toxins are produced in tentacles as precursors, which contain signal peptide, pro-part, and mature peptide. The main function of propeptide as proposed is the protection of sea anemone tissues from crucial damages by own toxins. Furthermore, there is hypothesis that propeptide promotes the toxin delivery into nematocysts. The Kunitz-peptide precursors, comprising the pro-part, have not been described yet. Kunitz-peptides are actively investigating due to the ability to exhibit a wide range of biological activities, such as analgesic, antihistamine, antifibrinolytic, anti-inflammatory and others, which indicate their therapeutic potential.

Here we revealed precursor of HCIQ2c1-peptide of sea anemone *Heteractis crispa* containing the short propeptide (5 a.a.) with Lys-Arg cleavage site between signal and mature peptides. The appearance of pro-part probably indicates the involving of Kunitz-peptides in venomous secret to enhance its toxic effect. We showed the recombinant HCIQ2c1 inhibits trypsin (K_i 5.2×10^{-8} M) and is capable to form tight complexes with chymotrypsin, kallikrein, cathepsin G

and elastase. Moreover, the peptide at concentration of 10 μM exhibits cytoprotective activity by decrease of neurotoxic effects of 6-OHDA and amyloid-β by 47 and 49.2% respectively. Thus, we showed at the first time the precursor of sea anemone Kunitz-peptide contains propeptide. Recombinant HCIQ2c1 interacts with serine proteases and exhibit cytoprotective activity that allow considering the peptide as potential leader compounds for pharmacological applications.

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P-004: Harnessing human monoclonal antibodies for neutralisation of dendrotoxins in a murine model

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Snakebite is a disease of the rural tropics, which causes death, disability, and destitution. One of the most feared venomous snakes in Africa is the infamous black mamba, *Dendroaspis polylepis*. *D. polylepis*' venom is comprised of a multitude of toxins, the most lethal and abundant of which fall within the families of dendrotoxins and alpha-neurotoxins. Currently, envenomings are treated with antivenom based on plasma-derived antibodies. While these antivenoms save lives, the safety, efficacy, and affordability of some products are suboptimal. Recombinant antibodies represent a therapeutic alternative. We are developing an antivenom based on recombinant, human, monoclonal antibodies of the immunoglobulin G (IgG) format, predicted to be improved in the aforementioned

parameters. Here, we present a subset of this work: The characterisation of the *in vivo* neutralising abilities of monoclonal and oligoclonal IgG mixtures against whole venom and venom fractions from *D. polylepis*. Dozens of IgGs were tested, and two cocktails comprising three and four IgGs, respectively, were designed based on toxin-specificity. These cocktails provided 100% survival in murine models of envenomation, even at low doses.

P-005: Cardiovascular collapse induced by *Echis ocellatus* venom: an *in vivo* and *in vitro* examination

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The carpet viper (Echis ocellatus) is one of the most medically important species of venomous snakes found in Africa causing thousands of deaths and permanent disability annually. E. ocellatus envenoming can result in cardiovascular collapse, venom induced consumption coagulopathy and major haemorrhage. The aim of this study was to investigate cardiovascular collapse due to E. ocellatus venom. Male Sprague-Dawley rats were anaesthetized (100 µg/kg ketamine/xylazine 10:1, i.p.) and cannulated for the recording of systemic blood pressure. In addition, small mesenteric artery myography experiments were conducted to determine in vitro cardiovascular activity. Venom at 10 µg/kg (i.v.) and 50 µg/kg (i.v.), but not 1 µg/kg (i.v.), caused acute cardiovascular collapse in anaesthetised rats as indicated by a rapid fall in blood pressure within 1-2 min without recovery. Prior administration of a single dose of venom (1 µg/kg, i.v.) or

heparin (300 UI/kg, i.v.) did not protect against the subsequent addition of 50 μg/kg (i.v.) venom. However, prior addition of two sequential doses of E. ocellatus venom (1+1 μg/kg, i.v.) or Brown snake (P. textillis) venom (2+2 μg/kg, i.v.) at 5 min intervals prevented cardiovascular collapse after the addition of 50 μg/kg of E. ocellatus venom. Venom (0.001-1 μg/ml) induced concentration-dependent relaxation in pre-contracted mesenteric vessels. The relaxation reached a maximum of 27.03 ± 13.96 % at a venom concentration of 1 μg/ml. This rather weak relaxation indicates that collapse is unlikely to be due to peripheral vasodilation. The prevention of acute cardiovascular collapse by the addition of small 'priming' doses, suggests a possible role for depletable endogenous mediators.

P-006: Assessment of toxicity of hydroponics *Stevia rebaudiana* Bertoni: Biochemical approaches

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Preclinical studies suggest pharmacological applications for stevia and their extracts because they are not toxic and exhibit several biological activities.Preliminary phytochemical studies showed the presence of stevioside, tannins, proteins, flavonoids, endemic microelements (I, Zn, Ge), minimal toxic elements (Pb, As, Cd, Hg) and absence of aflatoxin B1 (directly associated with liver cancer) and pesticide (DDT). Aflatoxin B 1 is the most common and most toxic one produced mainly by filamentous fungi asAspergillus flavus, A. parasiticus and A. nomius. The aspartat aminotransferase (AST)/alanin aminotransferase (ALT) (De-Ritis) ratio is an easily applicable blood test.Biochemical markers AST and ALT were used to assess the protective action of Stevia. The average value of ALT of normal rats was 10,42±0,8. Administration of Stevia at dose of 20mg/kg raised this value to 17,23±1,93. The average value of AST of normal rats was 10,02±0,7. Administration of Stevia raised this value to 17,19±2,76. De Ritis ratio was 0.99 in comparison to

the initial level (0.96), which indicates the absence of toxic effects following administration of ecologically pure hydroponic Stevia rebaudianaBertoni.

P-007: Brazilian *Bothrops diporus*, in fact a lineage of *Bothrops pubescens*: mitogenomic, venomic and ontogenetic studies

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The *Neuwiedi* complex is currently constituted by 8 species of venomous snakes geographically widespread through South America, including Brazil. The diversity hidden in this complex of snakes requires a deeper exploration in a taxonomic point of view as well as in the venom composition, for a better and systematic comprehension of the animal's biology. The current work brings a mitogenomic, venomic and ontogenetic studies of the *Bothrops diporus* in contrast to the *Bothrops pubescens*, both species from *Neuwiedi* complex and inhabitants of the Southern region of Brazil.

In order to access the composition of adult and juvenile venoms of both species, to characterize it and to evaluate ontogenetic changes, we used the Venomic approach. It consists in fractionation of the crude venom by RP-HPLC, followed by analysis by SDS-PAGE, *in gel* digestion and mass spectrometry. Our results showed that the venoms of both species are extremely similar, with an outstanding overlap not observed in the venom of other species of the complex. The intraspecific differences are mostly quantitative than qualitative, highlighting the balance of the two types of fosfolipases A2, Lys49 and Asp49, that clearly marks an ontogeny in these species. The adult venoms have high expression of Lys49 in relation to Asp49, while the juvenile venoms have similar expression of both Lys49 and Aps49.

The extreme interspecific venomic similarity caught our attention and led us to review the relation between these two species before other species of viperids. We assembled the complete

mitochondria of both and conducted phylogenetics studies. Our data revealed a phylogenetic proximity between these species not yet observed among other species of viperids, not even between different populations of same species.

For the first time we are showing a clear ontogeny inside the *Neuwiedi* complex, and in addition we are strongly suggesting that *B. diporus* is in fact a lineage of *B. pubescens*.

P-008: C-type lectin, hellercetin, negatively regulates melanoma cell adhesion and increases permeability

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The incidence of malignant melanoma is growing rapidly worldwide, and there is still no effective therapy. Additionally, this type of cancer is highly resistant to conventional DNA-damaging chemotherapeutics. Therefore, the search for novel tumor inhibitors and understanding the molecular pathways underlying chemoresistance is greatly warranted. Herein, we describe a novel C-type lectin called Hellericetin isolated from *C. o. helleri* venom using Cation Exchange Chromatography. Hellericetin had a molecular weight of about 21 kDa, and its identification was confirmed by Liquid Chromatography Mass Spectrometry (LC-MS/MS). Hellericetin inhibited ristocetin-induced platelet aggregation and Sk-Mel-28 cell adhesion to fibronectin with an IC_{50} of 125 nM and 7 μM, respectively. Interestingly, Hellericetin increased Sk-Mel-28 cell permeability by 41% compared to PBS controls. We found that pErk was significantly increased after Hellericetin stimulation, affecting cell morphology, but was not cytotoxic. Our results shed new light on the utility of C-type lectins for treating melanoma and suggest an undefined mechanism of action for cell adhesion, permeability, and ERK activation. In summary, C-type lectins show promising

potential as novel toxin therapeutics and research tools for the treatment and mechanistic understanding of diseases such as cancer.

P-009: Venom of jellyfish *Gonionemus Vertens* contains components against various types of cellular receptors

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Flash-like growth of jellyfish in coastal areas is a serious threat to human health, due to its venomous nature. Venoms of many jellyfish species are still completely unexplored and are of great interest in the field of toxinology. Various symptoms caused by jellyfish envenomation allow drawing conclusions about the venom of jellyfish as a promising source of new biologically active compounds that can be used in the development of new therapeutic drugs.

Jellyfish *Gonionemus vertens* is the serious threat to human health on the Far East coast of the Sea of Japan. Envenomation causes fever, myalgia, difficulty breathing, numbness of the limbs, in some cases paralysis. The composition of the venom and its components has not been studied yet. We tried to outline the pool of main cellular receptors which may be sensitive to venom's components.

In the course of this work, *G. vertens* venom was separated by gel filtration chromatography to seven fractions for biological activity investigation. One fraction showed high toxic activity on coastal crabs and decreased viability of mouse neuroblastoma Neuro2a cells. This fraction demonstrated 80% inhibition of labeled alpha-bungarotoxin binding to muscle-type Torpedo californica ray and human α7 nAChRs. As well this fraction included components showed 11%

inhibition of rat ASIC1a channel expressed in Xenopus laevis oocytes. Some other fractions showed activities against the rat TRPV1, mouse TRPV2, human TRPV3 receptors expressed in CHO cells in a Fluo-4–based intracellular calcium assay.

As a result we confirmed the multifunctional effect of the venom of *G. vertens* on mammals via different biological systems. The above-mentioned properties are of particular interest to this object and require further research.

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P-010: Snake's and arthropod's venom-induced pain-like behavior

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The evaluation of nociceptive behavior in mice during different venoms action and the investigation of the antinociceptive effect of *N*-Acyl amides and some analgesics was carried out.

"Biting/Licking of hind paw" method of estimating of nociceptive behavior in mice was used. *Macrovipera lebetina obtusa* (MLO), *Montivipera raddei raddei* (MRR) and *Daboya russelli russelli* (DRR) snake's venoms and *Mesobuthus eupeus* (ME), *Apis mellifera* (AM) arthropod's venoms were injected in hind paw (intraplantar) of mice. *N*-Acyl amides, analgesics, and cobra (*Naja naja oxiana*, NOX) venom were injected intraperitoneally.

MLO venom with inhibited phospholipase A_2 (by bromphenacyl bromide), induced less pain (about 2.8 times, $p<0.001$) but with inhibited metalloproteinases (by EDTA-Na_2) did not show significant difference with intact MLO venom in nociceptive behavior. Endocannabinoids had a different antinociceptive effect, from weak to strong: Arachidonoyl-Serotonine<Arachidonoyl-Taurine<Oleoyl-Taurine<Arachidonoyl-Ethanolamine <Arachidonoyl-Dopamine<Arachidonoyl-

Vanillin. Analgesics, Taurine and NOX venom, had antinociceptive effect from weak to strong: Taurine < Diclofenac < Metamizole < NOX venom.

Cannabinoids and analgesics show the antinociceptive activity against venoms. Arachidonoyl–Vanillin and NOX venom show the most effective antinociceptive action. Snake venom injection leads to pain-like behavior due to tissue and nerve injuries. Arthropods venom neuropeptides act on specialized channels and receptors. So there is a difference between snakes and arthropods venom action timing – the last one's venom injection cause expressed pain-like behavior from first seconds and help them to scare off predators, but for snake venoms, painfulness is additional, attendant effect.

P-011: Antinociceptive effect induced by a PnPP-19 derivative: new insights into venom peptides targeting opioid receptors

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Background: The peptide PnPP-19 (19 amino acid residues) is derived from the toxin δ- CNTX-Pn2a (48 amino acid residues) isolated from the venom of the spider *Phoneutria nigriventer*. PnPP-19 has been studied by our group as a new drug candidate to treat erectile dysfunction and pain. We showed that PnPP-19 induces antinociception and this effect seems to involve activation of opioid receptors and inhibition of neprilysin (NEP). derPnPP-19 is a new, synthetic and smaller peptide obtained from the primary structure of PnPP-19. Therefore, we intended to investigate whether derPnPP-19 could maintain the properties of PnPP-19 in the nociceptive pathway.
Methods: The Two-Electrode Voltage Clamp technique was used to evaluate whether derPnPP-19 could directly bind and activate human mu-, delta- and kappa-opioid receptors expressed in *Xenopus laevis* oocytes. To evaluate the antinociceptive effect of derPnPP-19, the paw pressure test in rats,

was conducted. derPnPP-19 was administered intraplantarly alone or with selective opioid receptors antagonists. *Ki* of derPnPP over NEP was determined using a fluorescence resonance energy transfer (FRET) substrate monitored in spectrofluorimeter.

Results: derPnPP-19- could directly and selectively activate the mu-opioid receptor subtype. The potency of activation induced by derPnPP (1 μM) was 90% of the response evoked by morphine (1 μM). EC50 value of derPnPP-19 for mu-opioid receptor yielded 13 nM. derPnPP- 19 (5, 10 and 20 μg/paw) induced peripheral antinociception. Only the specific mu-opioid receptor antagonist clocinnamox inhibited the antinociceptive effect of derPnPP-19 (20 μg/paw). derPnPP-19 could inhibit NEP and the *Ki* value obtained was 72 μM. **Conclusions:** derPnPP-19 maintained the antinociceptive effect of its precursor (PnPP-19), however, the derivative has a greater affinity to mu-opioid receptors. The data presented herein might be useful for the design of new opioid agonists.

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P-012: Honey *Apis Meliffera* bee venom modulate ovarian hyperstimulation syndrome by altering expression of vascular factors

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Aim and purpose: Ovarian hyperstimulation syndrome (OHSS) mostly occurs in women sensitive to endogenous gonadotropins, and women who undergo gonadotropin therapy in order to stimulate ovarian follicles to growth and maturation and to ovulation induction. This syndrome involves almost 20% of IVF-experienced women, and causes abnormal conditions through pregnancy, such as stomachaches caused by acts, and digestive abnormalities like intensive vomiting, reduction in urine rate, systemic edema, especially in feet, and some problems in the last months of pregnancy such as preterm labor, preterm rupture of the amniotic sac, and low birth weight. Honey Bee Venom (HBV) contains biologically active components possessing pharmaceutical properties. This study is

designed to assess the possibility of HBV application as an anti-inflammatory and anti-angiopathy therapeutic agent to suppress main inflammatory mediators such as serum IL-6 and ovarian follicles expression of COX-2 and VEGF in OHSS, an inflammatory disorder.

Method: We studied on 106 female rats (40-50 mg) in 6 groups of control, sham OHSS, HBV, OHSS, OHSS+HBV and OHSS+metformin, as positive control. All groups received 10 units of PMSG for 4 consecutive days and on the 5th day, 30 units of hCG were administered IP to induce OHSS. The control group received no treatment. Rats were then anesthetized and the ovaries were removed to examine the serological and immunological tests for IL-6, COX-2 and VEGF expression in all groups.

Results: Sociological data showed a significant reduction in peritoneal and ovarian vascular permeability, whereas, cholesterol and HDL increased significantly in HBV-treated groups compared to control and sham groups. Inflammatory feature of OHSS was modulated in HBV-treated OHSS group compared to OHSS, more effective than metformin. On the other hand, the results showed that HBV can prevent MDA and LDH production and also IL-6, COX-2 and VEGF overexpression in OHSS. Conclusion: The results of this study indicate that HBV can influence on the inflammatory feature of OHSS through angiogenesis factor reduction.

P-013: Effect of hydroponic *Teucrium polium* in ovariectomized rats

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There is compelling evidence that glutamate excitotoxicity is central to the progressive neurodegenerative processes. Glutamate receptors make up 90% of neurotransmission in the cortex. In the present study, we investigated the effects of hydroponic Teucrium polium on neuronal activity of temporal cortex during high- frequency stimulation (100 Hz for 1 second) of hippocampus in bilaterally ovariectomized rats. It has been found that the main biologically active compounds of hydroponic Tecrium polium are phenylpropanoid glycosides-verbascoside, poliumoside, teupolioside (up to 6 %) and flavonoids (up to 3 %). For the study were obtained the 50 % ethyl alcohol soluble extracts of wild, hydroponic (without furolactone acids) and soil Teucrium polium and the extract was then dried with rotary vacuum evaporator and were separated

benzene, ethyl acetate, chloroform-methanol (3:1) and aqueous fractions. The maximum tolerated dose of aqueous fraction of ethanol extract without furolactone acids showed no hepatotoxicity and ALT, AST levels and De Ritis ratio were within the normal range. Thus, Teucrium polium reduced OVX-induced neurodegenerative alterations in temporal cortex-hippocampus circuits.

P-014: *Phoneutria nigriventer* spider toxin PnTx2-1 (δ-Ctenitoxin-Pn1a) is a modulator of sodium channel gating

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Spider venoms are complex mixtures of biological active components with potentially interesting application for drug discovery or for agricultural purposes. The spiders of the genus *Phoneutria* inhabit forests of the neotropical region from Southern Central America throughout South America. These spiders are also known as the "armed spider" or "Banana spider". They are active hunters, relying on their fast acting and efficient venom for prey capture and defence. The spider *Phoneutria nigriventer* is responsible for a high number of envenomation with severe clinical manifestations in humans. A more efficient treatment requires a comprehensive knowledge of the venom composition and of the action mechanism of the constituting components. PnTx2-1 (also called δ-Ctenitoxin-Pn1a) is a 53 amino acid residues peptide isolated from the venom fraction

PhTx2. Although PnTx2-1 is classified as a neurotoxin, its molecular target remained unknown. This study describes the electrophysiological characterization of PnTx2-1 as a modulator of voltage-gated sodium channels. PnTx2-1 is investigated for its activity on 7 mammalian Nav channel isoforms ($Na_V1.1$-$Na_V1.6$ & $Na_V1.8$), 1 insect Nav channel from the German cockroach *Blattella germanica* ($BgNa_V1$) and 1 arachnid Nav channel from the mite *Varroa destructor* ($VdNa_V1$). Furthermore, comparison of the activity of PnTx2-1 and another *Phoneutria nigriventer* venom peptide, PnTx2-6, on $Na_V1.5$ channels reveals that this family of *Phoneutria* toxins modulates the cardiac Na_V channel in a bifunctional manner, resulting in an alteration of the inactivation process and a reduction of the sodium peak current.

P-015: The correlation of excitatory and depressor synaptic processes in pallido-nigral projection on the model of Parkinson's disease with protection by *Vipera raddei* venom

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On the 11 rats of Albino lines in three experimental series (in norm, on the model of Parkinson's disease – PD and with protection by Vipera Raddei - VR venom) the analysis of relative degree intensity of depressor and excitatory effects of 422 single neurons impulse activity of compact pars of substantia nigra (SNc) under high frequency stimulation of globus pallidus (GP) has been conducted, on example of diagrams of averaged spikes frequency, derived on the bases of raster of pre- and poststimulus manifestations of spike activity. In norm the expressed depressor poststimulus effects has been revealed, that confirms of GABAergic regulation of GP neurons activity, including from own intranuclear axonal collaterals. On the model of PD, compared to the norm, the increase of excitatory effects and its 2-multiple decrease with use of VR venom has been shown. On the model of PD the tendency of significant increase of prestimulus activity frequency of SNc neurons, preceding to poststimulus effects has been shown and its reduction using venom VR, but without achievement to norm. On the model of PD the sharp growth of poststimulus activity frequency of SNc neurons, accompanied by poststimiulus reaction has been revealed, and with use of VR venom - almost a threefold decrease of frequency and again without achievement to norm.

That's to be expected because powerful excitotoxicity, accompanying the neurodegeneration, with subsequent apoptosis and death of neurons. In conclusion, the use of venom VR promoted confrontation neurodegeneration.

P-016: Effect of premedication with subcutaneous adrenaline on the pharmacokinetics and immunogenicity of equine whole IgG antivenom in a rabbit model

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Subcutaneous administration of low doses of adrenaline is used to prevent the early adverse reactions (EARs) induced by snake antivenoms. We used a rabbit model to study the effect of premedication with adrenaline on the potential of antivenoms to exert therapeutic effects and to induce late adverse reactions. We found that premedication with adrenaline did not change the heart rate or blood pressure of normal rabbits previously sensitized with antivenom. Pharmacokinetics studies suggest that premedication with adrenaline does not affect the ability of the antivenom to exert the initial control of envenomation nor the susceptibility of rabbits to develop recurrence of antigenemia and envenomation. Our results also indicate that it is unlikely that premedication with adrenaline decreases the incidence of late reactions induced by the antivenom administration, although it reduces the extent of early reactions.

P-017: Type II toxins from sea anemone *Heteractis crispa* with various effects on activation and inactivation of voltage-gated sodium channels

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Voltage-gated sodium channels (Na_V) are key elements of cellular communication in a wide range of organisms, so this family of transmembrane proteins is a prime target for toxins found in sea anemones. All four structural types of sea anemone toxins presumably bind extracellular link within the fourth domain (site 3) disrupting channel inactivation. However, none of the type II toxins were characterized electrophysiologically in depth.

To test whether the type II toxins from Heteractis crispa, δ-SHTX-Hcr1f, RTX-III, and RTX-VI, were able to modulate Na_V channels, they were screened (at 10 μM) on mammalian, $Na_V1.1$ – $Na_V1.6$, $Na_V1.8$, and insect, $BgNa_V1$, $VdNa_V1$, channels expressed in Xenopus laevis oocytes. The increasing of the currents amplitude, of both fast and slow components, associated with an incomplete inactivation process and a significant positive shift of voltage dependence, of both activation and inactivation curves, was observed for insect channels and Na_V subtypes of CNS. Similar to previously observed data, δ-SHTX-Hcr1f, RTX-III, and RTX-VI were not phyla-selective (insect vs. mammalian) but showed some specificity. Thus, when both of the mammalian and insect subtypes of Na_V were treated by the same toxins concentration, δ-SHTX-Hcr1f, RTX-III, and RTX-VI had a more profound effect on the inactivation process and the current amplitude of insect channels. At the same time, IC_{50} values of Heteractis toxins obtained for insect Na_V channels substantially exceed those for mammalian Na_V. All observed effects of δ-SHTX-Hcr1f, RTX-III, and RTX-VI on Na_V currents are consistent with the previous characteristics of sea anemone toxins, including a different action on Na_V subtypes which amino acid sequences are completely identical within the region, responsible for toxin binding. These data suggest that the actual binding site is not limited by extracellular link mentioned above.

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P-018: Anti-angiogenic effects of the Macrovipera lebetina obtusa snake crude venom and obtustatin

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Macrovipera lebetina obtusa (MLO) is a poisonous snake in Armenia. Obtustatin represents the shortest known monomeric disintegrin, isolated from the snake venom of MLO, and is known to specifically inhibit α1β1 integrin. Its oncostatic effect is due to the inhibition of angiogenesis, which likely arises from α1β1 integrin inhibition in the endothelial cells. To explore the therapeutic potential of the MLO snake venom and obtustatin, we studied activity of obtustatin and MLO venom *in vitro*, by testing their efficacy in human dermal microvascular endothelial cells (HMVEC-D) and *in vivo*, using chick embryo chorioallantoic membrane assay (CAM assay). Our *in vitro* results showed thatobtustatin in comparison with MLO venom did not exibit cytotoxic activity in HMVEC-D cells in comparison to MLO venom. But *in vivo* results have shown that 4μg /embryo (90 μM) of obtustatin inhibited angiogenesis induced by FGF2 by 17% while MLO snake venom induced 22% reduction of the angiogenic index. The concentration of obtustatin in the crude MLO venom was 0.3 nM, which is 300.000 times less than the concentration of the obtustatin itself. Given this enormous difference in concentration, it is likely that some components of the crude venom contribute to the observed anti-angiogenic effect. Hypotheses will be ascertained to justify this action: components in the MLO venom may increase obtustatin efficacy or have independent but synergic anti-angiogenic activities.

P-019: Design and development of a new approach to formulate a hemostatic agent derived from the Iranian snake, *Echis Carinatus*

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Blood circulation plays a crucial role in the survival of vertebrates, including man. Nature offers a large variety of starting molecules, often highly specialized. Exogenous factors isolated from venoms of snakes that affect thrombosis and hemostasis have contributed to the development of diagnostic agents, research tools and life-saving drugs. Iranian saw-scaled snake (*Echis Carinatus*) venom is rich in proteins and peptides effective on the hemostaticsystem.

In the present study, we interrogated the complete sequence characterization of procoagulant peptides isolated from the most famous Iranian viper, *Echis Carinatus* by a combined approach of liquid chromatography coupled to MALDI mass spectrometry for the detection of even subtle differences of these peptides. We combined the procoagulant peptide isolated from *Echis Carinatus*venom withhydrogel.

In fact, this snake venom-loaded peptide hydrogel can be applied via syringe and conforms to the wound site resulting in hemostasis.The ability of mass spectrometry to identify and, increasingly, to precisely quantify thousands of proteins from complex samples can be expected to impact broadly on biology and medicine.

P-020: Fructose-induced neurotoxicity: electrophysiological study

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A significant association between fructose corn syrup in sweetened beverages consumption and increased risk of detrimental central nervous system effects has been recently reported. *Excess fructose intake* associated with *increased risk of* type 2 diabetes and diabetes-induced impairment in synaptic plasticity may contribute to the development of motor and cognitive defects in diabetic patients. In this study, we aimed to assess the electrophysiological indices of fructose-induced impairment of synaptic plasticity in the spinal cord and hippocampus. In vivo extracellular recordingfrom motoneurons to high-frequency stimulation (HFS) of sciatic nerve and hippocampal neurons to HFS ofthe entorhinal cortexin ratsfed on fructose-rich (50% body weight / volume) diet (for 9 weeks) was carried out. The analysis revealed an abnormal background spike activity, imbalances in excitation/depression responses to HFS and expression intensity of these responses. These parameters indicate fructose-induced toxic effects on synaptic properties of above-mentioned neuronal circuits. Our data focus on identifying the mechanisms of metabolic and electrophysiological changes in neuronal circuits seen in early stages of diabetes and may provide important clues about the cellular events responsible for diabetes-induced neuropathies.

P-021: α-Amylase inhibitors are major components of sea anemone *Heteractis magnifica* mucus

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Sea anemone mucus, due to its multiple and vital functions, is a valuable substance for investigation of new biologically active peptides, including pancreatic α-amylase inhibitors, which have a great pharmacological potential for the treatment of type II diabetes. The aim of this work is a searching of new pancreatic α-amylase inhibitors in mucus of sea anemone *Heteractis magnifica.*

Compounds of *H. magnifica* mucus were separated by multistage liquid chromatography and resulting fractions were analyzed by MALDI-TOF MS. Peptide maps constructed according to the

molecular masses and hydrophobicity showed presence of 326 peptides. Most HPLC fractions inhibited porcine pancreatic α-amylase, thus, α-amylase inhibitors along with proteinase inhibitors, pore forming toxins and neurotoxins are major components of *H. magnifica* mucus playing an important role in the successful existence of sea anemone. Magnificamide, the major α-amylase inhibitor of *H. magnifica*, was isolated and its amino acid sequence was determined (44 aa, 4770 Da). BLAST analysis of this sequence revealed only one sequence-based homolog (88.1%), which corresponded to helianthamide, α-amylase inhibitor from *Stichodactyla helianthus*.

With the help of genetic engineering approaches, a recombinant analog of magnificamide was obtained. Artificial gene encoding the peptide was cloned into pET32b vector and expressed in *Escherichia coli* as part of a fusion protein. The fusion protein was isolated from the cell lysate by metal affinity chromatography, hydrolyzed by endoproteinase, and then the recombinant magnificamide was purified by RP HPLC. The peptide inhibited porcine pancreatic and human saliva α-amylase. Thus, we obtained functionally active recombinant analog for further studies of its biological activity as potential drug for treatment of the type II diabetes.

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P-022: Antitumor efficacy of obtustatin in S-180 sarcoma mouse model

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Obtustatin, isolated from the Levantine Viper snake venom (*Macrovipera lebetina obtusa* -

MLO), is the shortest known monomeric disintegrin shown to specifically inhibit the binding of the α1β1 integrin to collagen IV. Its oncostatic effect is due to the inhibition of angiogenesis, likely through α1β1 integrin inhibition in endothelial cells. To explore the therapeutic potential of obtustatin, we studied its effect in S-180 sarcoma-bearing mice model *in vivo* as well as in human dermal microvascular endothelial cells (HMVEC-D) *in vitro*, and tested anti-angiogenic activity *in vivo* using the chick embryo chorioallantoic membrane assay (CAM assay). Our *in vivo* results show thatobtustatin inhibits tumour growth by 33%. The expression of vascular endothelial growth factor (VEGF) increases after treatment with obtustatin, but the level of theexpression of caspase 8 does not change.In addition, our results demonstrate that obtustatin inhibits FGF2-induced angiogenesis in the CAM assay. Our *in vitro* results show that obtustatin does not exhibit cytotoxic activity in HMVEC-D cells in comparison to *in vivo* results. Thus, our findings disclose that obtustatin might be a potential candidate for the treatment of sarcoma in *vivo* with low toxicity.

P-023: Transcriptomic and histopathological approaches to study *in vivo* venom response

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Introduction: High-throughput technologies have been poorly exploited in toxicovenomic field and thus the effects of snake venoms on global gene expression. **Objectives:** To investigate the local and systemic response to *Micrurus corallinus* venom in a mice model through the analyses of differentially expressed genes (DEGs) accompanied by histopathological studies. **Methods:** Swiss mice were injected in the gastrocnemius muscle with *M. corallinus* venom (50%LD_{50}) or saline solution. Total RNA from brain, kidney, liver, spleen, heart, diaphragm and gastrocnemius muscles were isolated at 8 and 24 hours after injection, and cDNA libraries were prepared and sequenced in an Illumina HiSeq1500 instrument. The set of DEGs, computed by DESeq2 and edgeR software,

were subjected to enrichment analyses with Metacore™. Moreover, samples of the muscle injected with venom were collected for histopathological analyses. **Results:** The highest number of DEGs (two-fold change cutoff, FDR ≤ 0.05) was found in the right gastrocnemius, local of venom inoculation. The enrichment analyses over these DEGs evidenced that most statistically significant networks and pathways were related to immune response, inflammation, chemotaxis and cell adhesion, mainly at 8 hours. In this context, several transcription factors, for instance: *Egr1*, *Fos*, *Junb*, *Myc*, and *Nfkb*, as well as, a myriad of membrane receptors and ligands were found up-regulated. The histopathological observations confirmed a leukocyte infiltrated in the necrotic muscle, probably due to the injury cause by PLA_2. Systemic effects were milder and detected mainly in the liver, indicating the IL-6 pathway as the most relevant one, with some up-regulated genes coding for acute phase proteins. **Conclusion:** Combining a modern technology, RNA-seq, and a traditional approach, histology, it was possible to get a comprehensive view of the molecular mechanisms underlying pathological effects in response to *M. corallinus* venom in a whole organism model.

P-024: Inactivation of *Macrovipera lebetina obtusa* Snake Venom by Enzymes of Multidrug Resistant *Pseudomonas*

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Pseudomonas are well known Gram-negative microorganisms with the wide diversity of enzymes, which define multidrug resistance and high adaptivity in terms of changing conditions of the environment. They can biodegrade both natural and synthetic toxic compound, xenobiotics like petroleum, etc. The level of proteolytic activity in the cell of these microorganisms is especially high. Snake venoms are very different by their ingredients, depends on several species and the areal of the snake. But the main destructive influence of venom to the human and animal organism after bites is caused by peptide and protein components with various molecular weight. The venom of

Macrovipera lebetina obtusa is a multicomponent system with various proteins, enzymes, toxic peptides and other substances with low molecular weight.

During this research, the ability of proteolytic enzymes of different multidrug resistant Pseudomonas strains to degrade components of Macrovipera lebetina obtusa snake venom was tested. There were used soil non-pathogenic strains of Pseudomonas from the National Culture Collection of microorganisms of the Microbial Depository Center of the SPC "Armbiotechnology" NAS RA.

As a result of experiments, it was found out that the activity of metalloprotease and phospholipase of Macrovipera lebetina obtusasnake venom in vitro was significantly decreased by the influence of bacterial enzymes of Pseudomonas aeruginosa 5249.

P-025: Serological, histopathologic and scintigraphic assessment of *Hemiscorpius lepturus* effects on renal dysfunction in rats

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Introduction:*Hemiscorpius lepturus* is one of the most dangerous scorpions of Iran leading to acute kidney injury (AKI) in minority of cases. The purpose of this animal study was to evaluate the predictive potential of scintigraphic method in acute kidney injury due to envenomation as soon as possible.

Methods: In two groups of animals each contained three rats, *Hemiscorpius lepturus* venom (1200µg/Kg) were injected intravenously via tail vein. At three hours and one week later, ^{99m} TC-DMSA (3mCi) was intravenously injected and renal scintigraphy were performed after an hour. Moreover, plasma levels of creatinine, sodium, potassium and blood urea nitrogen were measured. At the end of the study, renal tissues were excised and prepared to perform pathological evaluation after Hematoxylin and Eosin staining.

Results: All serological indices remained unchanged compared to control. Large number of glomerular fibrin thrombi with entrapped red blood cells and simplified tubular epithelium in dilated and ectatic tubules were seen in high power field (×100) four hours after envenomation, which reduced significantly one week later. In our scintigraphic study, there was a statistically significant differences ($p<0.05$) in kidney count rate per pixels (CRPP) in both acute and chronic phases compared to the sham group received normal saline (0.84±0.05 and 1.36±0.07 versus 1.7±0.05).

Discussion: It seems that serological parameters as the feasible indices could not be used to predict AKI in animal studies. On the contrary, for the first time, the results of this study introduces renal scintigraphy as an exceptional method to predict the occurrence of the AKI in *Hemiscorpius lepturus* envenomation.

P-026: Neurotoxic effect of *Macrovipera lebetina obtuse (MLO)* and *Montivipera raddei (MR)* snake venoms on rats Neuromuscular Junction (*NMJ*)

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According to literature data, venoms of some vipers besides hemorrhagic effect also have neurotoxic properties. The aim of this study is to inspect whether the venoms of *MLO* and *MR* have neurotoxic effects.

Neurotoxic effect at different periods of these venoms on *musculussoleus* of the rats was studied *in vitro* by the Electrophysiological Recordings method.*MLO* and *MR* venoms was diluted with physiological saline (0.9% NaCl, 1mg/ml and 1mg/2ml) to a final concentration of 10 µg/injection and 5µg/injection respectively. The target groups of the research has been divided into 3 groups: 1) control (soleus muscle without any venom intervention) 2) muscles that have been injected with *MLO* snake venom, and 3) muscles that have been injected with *MR* snake venom. Single excitation of *soleus muscle* generates chemically initiated action potential.

Latency of registered action potentials after electrical excitation of the nerve of *soleus muscle* in the control group was 0.38-3.02 msec (n=18). Their amplitude was 10-70mV(n=15). In *soleus muscle* injected with *MLO* venom there was observed significant increase of latency 0.61-6.19msec (n=7) and decrease of amplitude of potentials during 5-10 min 2,1-0mV. In case of *MR* venom we have observed increased latency for about 0.88-3.52msec (n=4) and decreased amplitude of action potential to 0-3.3mV (n=4) after 20 minutes.

As a sum, by comparing the data of effects of these two types of venoms, it can beconcluded that *MR* venom is acting considerably slower onNMJ and generates partial neuromuscular paralysis in *vitro*. In the case of *MLO* venom, the effect is seen after 10 minutes of injection and total paralysis is identified, but in the case of *MR* venom, after 20 minutes of injection, a small but yet noticeable action potential can be still identified.

P-027: Protective effects of curcumin against rotenone-induced Neurotoxicity

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Exposure of rats to the pesticide and complex I inhibitor rotenone reproduces features of Parkinson's disease, including selective nigrostriatal dopaminergic degeneration. Here, we examined mechanisms of rotenone toxicity using rat model. Curcumin is a naturally occurring phenolic yellow chemical isolated from the rhizomes of the plant Curcuma longa (turmeric), and is a major component of the spice turmeric. Curcumin has protective effects against rotenone-induced neural damage in Parkinson's disease (PD). The present study aims at providing new evidence for the validity of the rotenone rat model of PD by examining whether neuronal activity in the hippocampus is altered. Male albino rats were treated with rotenone injections (2.5 mg/kg intraperitoneally) for 21 days. We examined the effects of curcumin (200 mg/kg) on behavior and electrophysiology in a rat model of PD induced by rotenone. Motor activity was assessed by cylinder test. The electrical activity of neurons was measured in hippocampus. Rotenone causes significant reduction of neuronal activity. The results show that curcumin can improve the motor impairments and electrophysiological parameters and may be beneficial in the treatment of PD.

P-028: Effect of silver nanoparticles on peroxidase activity of *Linum ausriacum* L. *and Hypericum perforatum* L. callus culture

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In a detailed study of the synthesis of chemical nanoparticles (NPs), it was found that these particles could potentially pose a danger, including carcinogenicity, genotoxicity, cytotoxicity and general toxicity. The sizes of NPs of engineering nanomaterials are so small that they can pass through the skin, lungs and intestines with unpredictable consequences for human health. Effect of the silver NPs (AgNPs "AgBio-2", kindly provided by Dr. Ananyan), different concentrations on the morphology and antioxidant properties of *L. austriacum* suspension and *H. perforatum* shut cultures was studied.

In the first days of *L. austriacum* suspension cultures passing the exposure of high concentrations of AgNPs led to a change in the color of the flax cultures from green to yellowish-gray, and on the 7th day - to the restoration of color. Minimal inhibit concentration for NPs was 1,05mg/l. Under the influence of AgNPs, longer stems appear in *H. perforatum* culture than in control.

A culture medium pH study showed that AgNPs lead to pH drop that may be associated with oxidative stress. The effect of AgNPs leads to an almost twofold increase in the peroxidase activity in *L. austriacum* suspension cultures compared with the control. The effect of AgNPs on the peroxidase activity of *H. perforatum* shut cultures showed other results. The activity of peroxidase in callus cultures grown in the presence of NPs is reduced almost 4.2 times compared to the control. Peroxidase activity decrease during cultivation can be due to the synthesis of lignins, which together with cellulose compose the main structural components of the plants cell wall and play a major role in the plants adaptation to environmental conditions. The above may be the cause of the stems lengthening during the plants cultivation.

Thus it can be assumed that silvernanoparticles increase the stress resistance of callus cultures.

P-029: Purification and characterization of cysteine rich-secretory proteins (crisps) from the venom of the southern pacific rattlesnake (*crotalus oreganus helleri*): their role on blood and lymphatic endothelial cell permeability

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Cysteine-rich Secretory Proteins (CRiSPs) have long been recognized as ubiquitous components of many snake venoms, however, no clear explanation has been provided for the role they play in venoms. Some CRiSPs have been shown to inhibit ion channel activities and have major effects on cell signaling pathways in vascular endothelial cells. We speculate that CRiSPs, via combined effects on cell signaling pathways and ion channel activities, disrupt normal interstitial fluid dynamics adjacent to the snakebite, accelerating the transfer of the macromolecular toxins in the venom into the lymphatic circulation, which plays a critical role in venom absorption and distribution into the systemic circulation. The rapid delivery of these toxins into the circulation contributes to the acute effects of envenomation and the rapid incapacitation and death of the snake's prey. The goal of our study is to characterize the cellular and molecular basis for the effects of Hellerin, a newly identified CRiSP isolated from the venom of the Southern Pacific rattlesnake, on the function of blood and lymphatic endothelial cells. Crude venom was characterized by reversed-phase HPLC fractionation, followed by analysis of chromatographic fractions by SDS-PAGE and N-terminal sequencing. The N-terminal sequence of a 28 kDa protein band in fraction 13 was determined and identified as a CRiSP family. svCRiSPs were isolated and characterized from the snake Crotalus oreganus helleri. The preliminary results showed cytotoxicity to human umbilical vascular endothelial cells (HUVEC). CRiSPs will be further purified by cation exchange column and test their roles on human dermal blood and lymphatic endothelial cell permeability. Knowledge

gained from these studies will contribute to a new level of understanding of the pathophysiology of snakebite.

P-030: Retrospective documentation of a confirmed white-lipped green pitviper (*Trimeserus albolabris* gray, 1842) bite in the south-central hills of Nepal

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This case report represents a documentation of envenomation by White-lipped Green Pitviper (*Trimeresurus albolabris*) which is an arboreal pitviper species found in South and Southeast Asia. It causes the majority of venomous snakebites among pitvipers. Clinical features vary from asymptomatic to serious coagulopathy that may progress into life-threatening or fatal hemorrhage. However, there are sparse proven cases described in published literature. Further, no specific antivenom targeted to pitviper bites is available in Nepal. Here, we report a case of noticeable coagulopathic envenomation due to the proven *T. albolabris*bite in the hills of central Nepal and transfusion of fresh frozen plasma did not reduce coagulopathy. This study highlights the poor management of pitviper bites and urgent need of improvement in diagnosis, monitoring, and supportive care of *T. albolabris* bites in its distribution ranges and studying the effectiveness of Thai pitviper antivenoms for the treatment of *Trimeresurus* species bites in Nepal. Therefore, this case study contributes to improve pitviper bite management in Nepal.

P-031: Comparative analysis of impact of snake venom and stimulation of *pvn* on sympathetic-parasympathetic disbalance of organism

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In this fragment of studies were performed acute experiments on white non-linear rats. Changes in some parameters (HR, IVE, ITRS, SWB) by method of HRV mathematical analysis (MA HRV) the activity of neurons of the solitary tract nucleus (NTS) at the high-frequency (100 Hz) stimulation of hypothalamus paraventricular nucleus (PVN) or at intraperitoneal injection of venom (*Macrovipera lebetina obtusa*) LD50 were studied. A comparative analysis of the results showed a high degree (60%) of the sensitivity of NTS – the first switching relay of visceral impulses ascending through afferents of vagus nerve, which is the beginning of the body's homeostasis when applying the above mentioned stimuli. The tetanic stimulation of PVN resulted in a prolonged post-tetanic potentiation when injecting venom and significant increase in the spontaneous rhythm of the studied units and their rearrangement were occurred. The analysis of HRV indices revealed a striking similarity of the results obtained, which was expressed in a sharp increase in HR (control-320, PVN stimulation-371, venom-375, respectively). Almost a threefold increase in IVE and 2.5 times the degree of centralization of the regulation processes of ITRS is observed. The sympathetic-vagus balance (SWB) is shifted toward "sympatization". All this indicates a violation of sympathetic-parasympathetic equilibrium when super-strong stimuli are applied. Analysis of the investigated parameters with 15-minute intervals showed that when the poison is introduced, the homeostasis is disturbed immediately and central integrative structures are involved in the process to restore the sympathetic-vagus balance, however, the body's intoxication with LD50 venom leads to deep autonomic shifts (loss of heart rate with extra systoles, loss of breath, and etc). There is full non reactivity of the NTS neurons and the death of the animal at the 60-80 minute after injection. The issues of the impact of intoxication as a factor causing excessive stress and deepening the sympathetic-parasympathetic imbalance provoking stress are discussed. The ways of overcoming the development of stress pathology with a favorable outcome are considered.

P-032: Detection of *Salmonella spp.* in broiler chicken meat sold in retail markets of Yerevan, Armenia

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Foodborne salmonellosis is one of the most common sources of Salmonella outbreaks with a high impact on human health. Among other sources, animal origin products, in particular, contaminated chicken meat have been considered as one of the main vehicles of Salmonella infection. According to the National Center for Disease Control and Prevention of Armenia, more than 350 annual cases of salmonellosis were estimated, which were associated with a large number of hospitalizations. Moreover, as in recent years there is a tendency of increasing the consumption of chicken meat in Armenians' diet, new problems might arise regarding salmonellosis. Therefore, this study aims to determine the presence of *Salmonella spp.* in broilerchicken meat sold in retail markets of Yerevan.

In the frame of this research, 7 locally produced and 9 imported (3 samples per each) frozen raw broilerchicken meat samples were tested. The obtained results showed the presence of *Salmonella spp.* only in one sample of locally produced raw broiler meat. Among the imported samples (from Russia, Brazil, and Ukraine), only in one chicken meat sample (imported from Russia), the *Salmonella spp.* was detected. It should be mentioned, that in the territory of Eurasian Economic Union, the import of chicken meat from Russia to Armenia can be implemented based on declarations of conformity and without any additional testing. Meanwhile, the obtained results underline the need for microbiological surveillance testing.

Overall, the presence of *Salmonella spp.* in both imported and locally produced chicken meat, showed possibilities of cross-contamination in various sources either in a processing plant or until storage at retail level. Appropriate hygiene practices and cooking methods prior to consuming should be implemented in order to ensure chicken meat safety before ingestion.

P-033: Comparison of *Crotalus durissus* venom from captive specimens and the Brazilian Crotalic Reference Venom

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Snakes of the genus *Crotalus* are very important from the epidemiological point of view, once, although they account for only ~9% of ophidian accidents, they display the highest lethality among Brazilian snakebites (1.1%). In Brazil, the genus *Crotalus* is represented by a single species, *Crotalus durissus*. Crotalic venom can cause neuro and myotoxicity. The elucidation of these activities is important both for envenomation-related research and for the production of anti-crotalic sera. Besides, by WHO recommendation, it was established the use of the Brazilian Crotalic Reference Venom (BCRV) as a standard for the biological activity assays of Brazilian snake venoms. Thus, since 1987 the National Institute of Quality Control in Health and the serum producers' Brazilian laboratories have used the BCRV. For the preparation of the reference venoms, the first extractions of snakes that have just arrived at the Butantan Institute are used. However, a gradual decrease in the number of snakes donated to the Butantan Institute has been observed, compromising the variability of the venoms used to produce the BCRV. So, the aim of this study is to compare the venoms from snakes born in Laboratory of Herpetology of Butantan Institute and the BCRV in order to verify the possibility of using these venoms in the composition of the BCRV. For this, tests of PLA_2 and LAAO activity, 1D electrophoresis, Western blotting and minimum coagulant dose (MCD) were made. Both activities, PLA_2 and LAAO, were 57% and 10% lower, respectively, in BCRV than in venom of captive snakes. However, Western blotting and protein electrophoretic profiles obtained by 1-D were similar. The MCD was higher in BCRV, displaying the higher coagulant activity of captive snake's venom. Concluding, the venom of captive snakes is very similar to BCRV, with differences between activities, possibly due to the long storage time of the BCRV in comparison to venom of captive snakes.

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P-034: A novel toxoid Phospholipase D1 from Iranian Hemiscorpius lepturus scorpion and immunogenicity studies in BALB/c mice

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Hemiscorpius lepturus (H. lepturus) is one of the most dangerous scorpions and the most medically important scorpion in Iran. The clinical signs of *H. lepturus* envenomation, including dermonecrosis, hematuria, renal failure and early death, are attributed to phospholipase D activity. This study was conducted to develop a novel recombinant phospholipase D1 (rPLD1) toxoid and investigate its immunogenicity and protective effects against the lethality of *H. lepturus* venom. The lethal protein recombinant phospholipase D1 was expressed from the PLD *H. lepturus* venom gland. The rPLD1 toxin was converted into a toxoid (the first toxoid of *H. lepturus* PLD) with a 0.25% concentration of formalin and stored for ten days at room temperature. In the toxicity test, the lethal activity of recombinant phospholipase D1 was fully inhibited. When it reached up to 3 times higher than the maximal effective concentration of the purified toxin (11.1 µg), rPLD1 toxoid was used. The sphingomyelinase activity was inhibited when up to 5.4 times of the LD100 of the purified toxin (20 µg), toxoid was used. It was then used to produce an antibody in BALB/c as an antigen and the mice were then challenged with rPLD1 toxin and the whole venom. The immunogenicity of rPLD1 toxoid was evaluated and the maximum titer of the raised antibodies was determined by ELISA assay. The optimum titer for anti-rPLD1 toxoid sera was obtained at the third intraperitoneal injection of rPLD1 toxoid, and a high titer was reached at the fourth injection in the mice. This toxoid increased the amount of antibodies and produced a protective antiserum against the whole venom of *H. lepturus* and rPLD1 toxin. The *in-vivo* test results showed that the mice were completely resistant against 200 times the LD100 of recombinant phospholipase D1 and the whole

venom of *H. lepturus*. To conclude, rPLD1 can be used in toxoid form as an immunogen in the production of a new generation of neutralizing antibodies against the lethality and toxicity of *H. lepturus* whole venom.

P-035: Action of *Macrovipera lebetina obtusa* venom in hippocampal neurons in the Alzheimer's disease rat model

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Alzheimer's disease (*AD*) is the most common *neurodegenerative disease*. The amyloid β peptide (Aβ) is a critical initiator that triggers the progression of AD. To date, several groups of drugs have been developed for AD that affect the formation of Aβ. Of great importance for neuroprotection are snake venoms. Macrovipera lebetina obtusa (MLO) is one of the most poisonous snakes in Armenia.

A comparative study of the morphofunctional state of the rat hippocampal cell structures by intracerebroventricular administration of Aβ 25-35 combined with the administration of MLO was performed. The characteristic morphological sign of hippocampal Aβ-induced neurodegeneration is a sharp decrease in phosphatase activity. With the systematic introduction of small doses of MLO venom after injection of Aβ 25-35 in all fields of the hippocampus the size and shape of the cells are restored, granulation appears around the perikaryon and processes, which is typical for primarily irritated neurons, which are on the way to recovery.

The results of the studies suggest that the observed positive structuralchanges of neurons, an increase in metabolism, an increase in the density of neurons and enhancement of Ca^{2+} -dependent phosphorylation determine cell survival. Thus, small doses of this venom exhibit obvious neuroprotective effects.

P-036: Retrospective evaluation of *Micrurus fulvius* (Eastern coral snake) envenomation and the use of mechanical ventilation in dogs and a cat: 8 cases (2011-2016)

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Objective – To describe the use of mechanical ventilation in the management of Eastern coral snake envenomation in dogs and a cat.

Design – Retrospective study (2011-2016)

Setting – University teaching hospital

Animals – Seven dogs and one cat receiving mechanical ventilation for ventilatory failure secondary to Eastern coral snake envenomation.

Interventions – All animals underwent mechanical ventilation, with seven patients also receiving either a $F(ab')_2$ or IgG coral snake antivenom.

Measurement and Main Results – The medical records of eight animals (seven dogs, one cat) that received mechanical ventilation following Eastern coral snake envenomation were reviewed. Data collected included signalment, if the snake bite was witnessed, time to veterinary assessment, physical and laboratory characteristics at presentation, clinical course during hospitalization, management including antivenom administration, mechanical ventilation settings, duration of ventilation, length of hospitalization, cost of care, and survival to discharge. The mean animal age was 4±3.2 years. Median time to onset of clinical signs was 30 minutes (range 5-240 minutes). Coral snake antivenom was administered to 7 of the 8 animals following presentation [median 30 minutes (range 5-90 minutes)]. All animals had progressive hypoventilation and received positive pressure ventilation, specifically volume controlled, synchronized intermittent mandatory ventilation with pressure support. The median duration of MV was 58 hours (range 25-84 hours) and the median duration of hospitalization was 8.2 days (range 6-11 days). Ventilator associated complications occurred in 8 of 8 animals, but overall outcome was excellent with 7 of 8 surviving to discharge.

P-037: How to reduce the tooth decay caused by microbial toxins?

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There are typically over 70 different types of bacteria in the mouth and most of them occur naturally, doing no harm to the person. There are, however, bacteria that can contribute to dental decay and periodontal (gum) disease in particular. They often cover parts of the cheeks and back of the throat, but they can live in between all the bumps and ridges found on the tongue. Streptococcus mutans, is the bacteria identified the most with tooth decay, and is present in all areas of the mouth. For dental decay to occur, according to Britannica, the normal presence of S. mutans in the mouth has to make contact with sucrose or sugar-containing products. This causes S. mutans count to increase in number and secrete acids and similarly harmful products that attack teeth's enamel resulting in decay.

Two other bacteria, Treponemadenticola and Porphyromonasgingivalis, also play a role in periodontal diseases and eventually cause the teeth to loosen.

To prevent tooth decay, it is first necessary to protect the teeth from mechanical damage such as heat and sudden cooling, breaking nuts, etc. Proper brushing, flossing and using antibacterial rinses for gum health can reduce the number of bacteria that build up in specific spots between the teeth and along the gum line. Proper training of teeth health to parents and children has a significant role in reducing dental injuries. Periodic examination of the teeth also helps to significantly reduce the damage and the development of dental damage.

P-038: Neurotoxic effects of cortisol on the brain in patients with the first episode psychosis

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Recent studies confirm the presence of hyperactivity hypothalamic-pituitary-adrenal (HPA) axis in patients with the first episode psychosis (FEP). The biomarker of the stress response is significantly elevated level of cortisol, however the prolonged exposure to increased doses is neurotoxic, which has a damaging effect on the brain. The article presents data on the study of levels of cortisol and dehydroepiandrosterone-sulfate (DHEA-S) in 33 patients with FEP, taking into account the severity of psychopathological symptoms compared with the 34 healthy controls. The severity of psychopathology was evaluated using the Positive and Negative Syndrome Scale (PANSS). Our results showed that patients with FEP had increased activity of HPA-axis, which is manifested in a significant increase in background levels of cortisol in women ($p<0.02$) and in men ($p<0.00$) in the group with expressed by psychopathological symptoms by comparison with the control group. The level of DHEA-S was also higher, but the significance of differences with the control group was found only in men ($p<0.02$) in the group with less pronounced psychopathological symptoms, which confirms the neuroprotective function of DHEA-S to compensate for neurotoxic effects of cortisol on the brain.

P-039: Three-finger toxin Mambalgin-2 from *Dendroaspis polylepis* venom reduces the growth of glioblastoma cells by inhibition of ASIC1a channels

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In high-grade glioblastomas ASICs subunits and other members of the ENaC/Deg receptor family form the chimeric channels. Inhibition of these channels by amiloride and spider toxin PcTx1 leads to decrease of the glioblastoma cell growth and invasion *in vitro* and *in vivo*. Recently, two peptides called Mambalgins have been isolated from venom of *Dendroaspis polylepis*. Mambalgins belong to the family of three-finger toxins and are potent inhibitors of ASICs.

Here, we tested the action of the recombinant analog of Mambalgin-2 on the rat ASIC1a channel, expressed in *Xenopus laevis* oocytes and showed that recombinant Mambalgin-2 inhibits ASIC1a with half-maximal inhibitory concentration (IC_{50}) of 142 ± 20 nM. Mutant variants of Mambalgin-2 with Leu/Ala substitutions of the residues in the position 32 and 34, which predicted to impair affinity towards ASIC1a channels, demonstrated decreased ASIC1a inhibitory activity. Mambalgin-2 mutant L32A showed almost complete loose of activity, while L34A mutant retained about 30% of the recombinant toxin activity.

Here we showed that recombinant Mambalgin-2 inhibited the growth of human glioblastoma cells U251 MG up to 61.25 ± 1.2 % of the control with EC_{50} ~ 0.6 ± 0.01 nM, while L32A and L34A mutants did not inhibit proliferation of the cancer cells.

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P-040: Arginine derivatives of dicarboxylic acids from toad venom — new agonists of ionotropic γ-aminobutyric acid receptors

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The venoms of the toads from the *Bufo* genus contain a large number of biologically active compounds of different chemical nature. At present, some of them have been isolated and

characterized. This is a powerful cardiac glycoside bufadienolide containing lactone ring in the structure. Other example is serotonin analogue bufotenine, which interacts with the serotonin receptor of 5-HT3 type.

We studied the venom of common toad *Bufo bufo* and found a new family of biologically active substances - the arginine derivatives of dicarboxylic acids. These substances were isolated from the venom using gel-filtration and reverse-phase chromatography. Analysis of their structures by mass spectrometry and nuclear magnetic resonance reveal that these are suberylarginine, pimeloylarginine, and adipoylarginine. The biological activity of the isolated compounds was studied by electrophysiology using whole-cell patch-clamp and HEK293 cells transiently expressing γ-aminobutyric acid receptor type A (GABAA) composed of α1β3 subunits. All three substances manifested a capacity to activate GABAA receptor. More detailed studies showed that they are partial agonists, the pimeloylarginine being the most active. Interestingly, free arginine did not activate GABAA receptor.

Thus, we have shown that the arginine derivatives of dicarboxylic acids are agonists of GABAA receptor.

This work was supported by RFBR 18-04-00844 grant.

P-041: Determination of scorpion venom LD50 of *Apistobuthus susanae* species in Khuzestan province; (Southwest of Iran)

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Scorpion stings are a common medical problem in the many countries in all of the world. Also this problem is there in the south and south western provinces of Iran including Khuzestan. Adequate assessment of scorpion venom LD50 is an important step for accurate evaluation of antivenom sera potencies and the optimization of serotherapy. The aim of the present study was to determine the LD50 of one species of Khuzestan scorpion.

Samples of *Apistobuthus susanae*scorpion species were collected from sandy hillsaround the city of Ahwaz capital of Khuzestan province using UV light technique overnight. Venom was obtained from scorpions by electrical stimulation of telson. Toxicity was determined after injecting intravenously (IV) the venom to albino mice (18-20grams) and calculating LD50 by Spearman-Karber method and Prof.Chi software.

The percentage of protein venom was calculated as 5.4mg /ml using Lowry method. The Lethal dose (LD50) of scorpion venom was determined as 203 µg in albino mice 18-20 grams. The regression line of mortality probits was planted using 2013Excel.Squared R was determined as 0.92.

P-042: Investigation of the action of silver nanoparticles on the biosynthesis of podophyllotoxins of *L. austriacum* suspension cultures

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Long-adapted suspension cultures of *L. austriacum*, were used as a model system for studying the mechanisms of action of silver nanoparticles (“AgBio-2”, kindly provided by Dr. Ananyan), having a spherical shape with a diameter of 2-18 nm in DSS was used as the stabilizer. Silver nanoparticles and DSS in a concentrations 1.05; 2.1: 3.1; 4.2 µg / ml were added to a liquid culture medium containing MC salts, vitamins, phytohormones BAP and NAA. The cultures were incubated on a shaker in a climatic cupboard (IRC Israel) under continuous illumination. Determination of the relative content of Ptox, 5-mPtox, de-Ptox, α- andβ -peltatins in L. austriacum cell cultures was conducted by HPLC on an HPLC-Termo Quest (Termo Quest, Germany) using Spherisorb ODS columns -2 ("Sigma", the USA).

At the first time we investigated the effect of silver nanoparticles on the biosynthetic potencies of callus cultures of *L. austriacum*. The effect of high concentrations of silver nanoparticles and stabilizer in the first days of passions leads to a change in the color of the flax cultures from green to yellowish gray, and on the 7th day to the restoration of native color. Despite this, the next days of cultivation lead to a decrease in biomass and protein content, with the manifestation of aging cultures. The medium containing 1.05 μg / ml nanoparticles was optimal for growth and biosynthesis of podophyllotoxins of callus cultures. Cell cultures of *L. austriacum* on medium MC-BN, accumulated 5 mPtox, Ptox, α- and and β-peltatin as the main products. Silver nanoparticles (1.05 μg / ml) increase the synthetic potencies of callus cultures with increasing of all lignan's concentration 1.4 - 1.6 times. Thus, silver nanoparticles can be used as a tool for the metabolic regulation of directed biosynthesis of specific secondary metabolites, in particular, the podophyllotoxins**.**

P-043: A forward to optimization of antivenom therapy: An *in vivo* study upon the effectiveness of the antivenom against early and delayed nephrotoxicity induced by the venom of the Iranian scorpion *Hemiscorpius lepturus* in rat

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The aim of the present in vivo study was to identify the optimal effective dose, the most favorable time and the route of administration of the available polyvalent scorpion antivenom against the toxic effects induced by *Hemiscorpius lepturus* (H. lepturus) venom in rat. The end point for assessment included measurement of alanin-amino-peptidase (AAP) and N-acetyl-b-d-glucosaminidase (NAG), biochemical urine analysis and histopathological assessment. The results

showed that a single subcutaneous 50µg of the venom produced significant increase in the AAP and NAG enzyme activity, urinary biochemical parameters and induced histopathological structural abnormalities in the renal system. The optimaleffective co-administered dose of the antivenom was 0.5 ml, which when administered 1 and 2 h of envenomation by intravenous (IV) and subcutaneous (SC) routes respectively producedsignificant protection against these toxic effects. Prudently, the significance of these findingsneed to be assessed in further clinical studies.

P-044: Toxin-like action of human secreted proteins SLURP-1 and SLURP-2 on epithelial tumor cells

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Activation of non-neuronal α7-nAChRs by nicotine or its derivatives (nitrosamines) could stimulate the development and progression of smoking related cancers. Downstream events of the α7-nAChR signaling influence cell proliferation, angiogenesis, EMT and migration of cancer cells. This makes α7-nAChRs a promising molecular target for the treatment of various types of cancer. Inhibition of α7-nAChRs by snake α-neurotoxins suppresses tumor cells proliferation, but α-neurotoxins are highly toxic and could not be used for systemic cancer treatment.

Previously we showed that recombinant human proteins SLURP-1 and SLURP-2, sharing structural homology with α-neurotoxins, inhibit α7-nAChRs. Comparison of the antiproliferative activity revealed that SLURPs significantly more effectively suppress the growth of epithelial cancer cells than α-bungarotoxin. For further evaluation of antiproliferative properties of SLURPs we tested them on multicellular spheroids, an *in vitro* 3D model of solid tumors. Multicellular spheroids were obtained from lung carcinoma A549cells, epidermoid carcinoma A431cells, and breast carcinomas SKBR-3 and MCF-7 cells. The effect of SLURP-1 and SLURP-2 on viability of multicellular spheroids was studied using MTT test. We found that 48-hour treatment with SLURP-1 inhibits the growth of multicellular spheroids derived from A549 cells to 80.3 ± 2.7% of control

(EC_{50} ~ 0.02 ± 0.001 nM), from A431 cells to 74.6 ± 3.2% (EC_{50} ~ 0.12 ± 0.05 nM), and from SKBR-3 cells to 62.4 ± 8.5% (EC_{50} ~ 0.02 ± 0.05 nM). SLURP-2 demonstrates an inhibitory activity only on spheroids derived from A431 cells. Both SLURPs have no significant inhibitory effect on the growth of MCF-7 spheroids. Presented results supplement the data obtained for epithelial cancer cells in monolayers and reveal new details of SLURPs antitumor activity.

The study was supported by the Russian Science Foundation (Project № 17-74-20161)

P-045: Detection of the binding sites for conotoxins RgIA and GeXIVA with two cholinoreceptor models.

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α-Conotoxins are the neurotoxic peptides from poisonous marine snails of Conus genus. They are useful tools in the isolation and structural/functional studies of nicotinic acetylcholine receptors (nAChRs). High affinity and selectivity of the conotoxins for different subtypes of nAChRs make them promising source for new drugs design. α-conotoxin RgIA analog and αO-conotoxin GeXIVA are the hopeful analgesics for the neuropathic pain treatment.

Both conotoxins RgIA and GeXIVA , blocking neuronal α9/α10 nAChR are characterized by a high content of arginine residues (4 and 9, respectively). Typically, α-conotoxins bind to the orthosteric sites, but αO-conotoxin GeXIVA attaches allosterically in the electrophysiological experiments on α9/α10 nAChR. According to the X-ray analysis, α-bungarotoxin (αBgt) disposition in the α9 subunit is similar to that in complex with the acetylcholine-binding protein (AChBP). Therefore we studied the binding sites of these conotoxins on two receptor models – extracellular domain of α9 nAChR (α9 ECD) and AChBP *Aplysia californica*. In the competitive radioligand assays with iodinated α-bungarotoxin (^{125}I-αBgt), we found that ^{125}I-αBgt was displaced by both conotoxins of

the α9 ECD. However, IC50 values were in the micromolar range, whereas IC50 values deduced from electrophysiology experiments on the full-size α9/α10 nAChRs were in the nanomolar range. We proved that this difference might be due to non-specific binding of these arginine-rich conotoxins to the Ni2+-NTA-agarose used for immobilization α9 ECD. Since immobilization AChBP does not require Ni2+-NTA-agarose, we tested both conotoxins on this model. The RgIA globular isomer was more efficient than the ribbon one, whereas all three GeXIVA isomers had similar potencies. Our results suggest the conotoxins attachment to the AChBP orthosteric site.

P-046: The protocol of choice for treatment of snake bite

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The aim of the current study is to compare three different methods of treatment of a snake bite to determine the most efficient one. To unify the protocol of snake bite treatment in our center, we retrospectively reviewed files of the snake-bitten patients who had been referred to us between 2010 and 2014. They were contacted for follow-up via phone calls. Demographic and on-arrival characteristics, protocol used for treatment (WHO/Haddad/GF), and outcome/complications were evaluated. Patients were entered into one of the protocol groups and compared. Of a total of 63 patients, 56 (89%) were males. Five, 19, and 28 patients were managed by Haddad, WHO, or GF protocols, respectively. Eleven patients had fallen into both GF and WHO protocols and were excluded. Serum sickness was significantly more common when WHO protocol was used while 100% of the compartment syndromes and 71% of deformities had been reported after GF protocol. The most important complications were considered to be deformity, compartment syndrome, and

amputation and were more frequent after the use of WHO and GF protocols (23.1% versus 76.9%; none in Haddad; *P* = NS). Haddad protocol seems to be the best for treatment of snake-bitten patients in our region. However, this cannot be strictly concluded because of the limited sample size and nonsignificant *P* values.

P-047: The Phytochemical Analysis Of Bulbous-Rooted Chervil (Charophyllum Bulbousum l.) Herb Growing Wildly In Different Regions Of Armenia

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In Armenia from ancient times have been used a number of wildly growing plants such as bulbous-rooted chervil (Chaerophyllum bulbosum L).The goal of this work is phytochemical analysis of bulbous rooted chervil's herb growing wildly in RA flora, as a source of biologically active compounds and minerals. As a material for the research served the overground part of bulbous-rooted chervil ,harvested on may-june, 2010, from different regions of RA.

The essential oil was obtained by hydro-destillation method and its chemical composition was determined by gas chromatography- mass spectrometry (GC-MS) method .The amount of heavy metals was determined by thermal- emission method.The analysis of the volatile fraction of Charophyllum bulbousum L. growing wildly in Aparan demonstrated the presence of at least 36 constituens and the amounts of only 15 components exceed 1% .The following major components were found; β-Caryophyllene epoxide, 6-[91E0-1,3-Dimethyl-1,3-butadienyl]-1,5,5-trimethyl-7-oxabicyclo[4.1.0]hept-2-ene, Pulegone, 3-Methyl-5propylonane, Eucalyptol, β-cis-Caryophyllene , l-Menthone , Dodecane , Spathulenol , (+)-valeranone, Heptaethylene glycol monododecyl ether etc. In all samples high amounts of Ca and Mg were present. Highest amounts of Si(15500mg\kg), Al(6510mg/kg), Mn(49,6mg/kg), Fe(3720mkg/kg),Cr(1,55mg/kg), Ni(6,51mg/kg), Zr(6,51mg/kg), Ti(279mg/kg), Va(8.68mg/kg) were found in the general ash of Aparan sample. P highest amount was found in the general ash of Kapan sample (1880mg/kg). Cu amount was almost the same in all

samples. And the highest amount of Pb was found in the general ash of Eghegnadzor sample (4,5mg/kg).

From 36 components of essential oil only 9 are of terpenoid structure. In all samples Pb amount exceeds maximal permissible density (Pb<0.5mg/kg).

P-048: Entamoebas as models for the structural and functional consideration of liposomes and phospholipases C in eukaryotes

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Cytopathogenic effects of host target cells by Entamoebas are rapid, contact-dependent events that appears by phospholipases C. By means of light, electron-microscopy, cryo-ultramicrotomy, biochemical methods it would be revealed the developmental, structural-functional new features of Entamoeba.

The aim of the electron microscopy study was, in addition to the ultra-cytochemical methods, to determine the localization of phospholipases and acid phosphatases in cells of *Entamoeba histolytica* and cholesterol-lecithin complexes containing liposomes. The study of the cholesterol-digitonin complex in cells of Entamoeba after enrichment of their cultures with cholesterol made it possible to reveal of cholesterol in the form of local thickening of membranes, monolamellar and multilamellar liposomes.

The results of the electron microscopic observations indicate the cholesterolization of the pathogenic Entamoeba's' membranes grown on cholesterol media containing liposomes, phospholipase C activity by the reaction product in the form of electron-dense precipitate was found

on the surface of the plasma and phagosome membranes of the cell of Entamoeba, which contribute to the cytopathogenic effects upon interaction with the host cells.

P-049: The Kunitz-type HCRG peptides from the sea anemone *Heteractis crispa* possess Kv channel toxicity

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Recently the two peptides isolated from the sea anemone Heteractis crispa, HCRG1 and HCRG2, belonging to the new H. crispa Kunitz-type HCRG subfamily, have been found to be potent serine protease inhibitors against trypsin and α-chymotrypsin (Ki ~ 10^{-8} M). Furthermore, they have possessed an anti-inflammatory activity, reducing tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) secretions, as well as proIL-1β expression in lipopolysaccharide (LPS)-activated macrophages.

The peptides have shared high homology of amino acid sequences and 3D structures with those of SHPI-1 Kunitz toxin from the sea anemone Stichodactyla helianthus and dendrotoxins (DTxs) from snakes of the genus Dendroaspis (family Elapidae). SHPI-1 and DTxs have been reported to block voltage gated potassium channels at micro- and nanomolar concentrations.

In this work we present the study of structure-function relationships in terms of HCRG1 and HCRG2 blocking effects on Kv1.1 channel. Electrophysiology experiments revealed that HCRG2 blocks rKv1.1 channel tenfold more potently than HCRG1 does (12.6±1.72, 142.6±28.1 nM, respectively). The molecular modeling approach including homology modeling, protein-protein docking with following MD simulations allows us to disclose the fact that despite the high structural homology with only point substitutions these toxins accommodate distinct orientations in their complexes with the channel. The patterns of functionally important residues of HCRG1 and HCRG2 for the channel blocking were recognized as well as a significant role of N-terminal Arg1 residue in Kv1.1 binding was clarified.

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P-050: Effect of amino acids near the RGD sequence on binding activities between αIIbβ3 integrin and fibrinogen in the presence of RGD-containing synthetic peptides from elegantin and angustatin

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Elegantin and angustatin, which were isolated from the snake venoms of Protobothrops elegans and Dendroaspis angusticeps, markedly inhibit binding between platelet integrins and fibrinogen via the Arg-Gly-Asp (RGD) sequence. Angustatin, which is a three-finger toxin containing the RGD sequence, inhibits platelet aggregation almost ten times more strongly than disintegrin isolated from the venoms of Viperidae and Crotalidae. The RGD sequences of both polypeptides are located at the top of hairpin loops, and the composition of the RGD loop is very important for binding to integrin.

We investigated the effects of synthetic RGD loop peptides from angustatin and elegantin on ADP- or collagen-induced platelet aggregation and αIIbβ3-fibrinogen binding. Synthetic angustatin (PRGDMP)-type peptides inhibited platelet aggregation more strongly than elegantin (ARGDDX)-type peptides. In particular, the cyclic angustatin peptide (CPRGDMPC) inhibited ADP- and collagen-induced platelet aggregation at least 10-50 times more strongly than the other peptides. The cyclic angustatin peptide (CPRGDMPC) was also the strongest inhibitor of binding between αIIbβ3 and fibrinogen, the IC_{50} of this peptide was approximately 2.58 μM [1)].

We suggest that cyclic angustatin peptide (CPRGDMPC) is useful for research on integrin-related proteins.

P-051: Phytochemical analysis of *Valeriana cardiola L.* rhizomes and roots cultivated in Zovuni region of Armenia

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Valeriana officinalis is the species most commonly used in northern Europe and still retains its official pharmacopoeial status although it is most commonly encountered as an ingredient of herbal medicines.

The aim of this study is on the base of phytochemical and integrated research creating pharmacologically active agents, in order to develop methods for standardization *V.cardiola*L. As a material served rhizoma cum radicibus, obtained from the *Valeriana cardiola L.*, cultivated in Zovuni region in the second half of September in 2015.

The qualitative analysis of essential oil was carried out by the gas chromatography method. The study was carried out on gas chromatograph with mass-selective spectrometer from "BRUKER" company (USA), an OPTIMA-FEAP-0.25mkm, 60m x 0.25mm ID was used, boiling temperature 220C, temperature gradient 50C (2min.), heated up to 250C (2.50C / min.) (duration: 5min.). Thecompounds were identified according to NIST data.

For the first time chemical composition analysis of volatile oil obtained from valerianacardiola rhizome and root raw materialwas carried out by GC-MS method. In the result of analysis 71 compounds; monoterpenes, sesquiterpenes and aromatic substances of non-terpenoidal structure are determined in valerian volatile oil. The analysis showed that in volatile oil the prevailing are the components of the 2 groups:sesquiterpenes (65.34%) and monoterpenes (31.12%).

As the analysis showed the major part of 71 determined compounds were derivatives of bornyl and myrtenol, neocloven and valeranone together with valepotrates demonstrate sedative and spasmolytic effects of Valerianacardiola L. rhizome and root raw material. In volatile oil determined compounds (more than 1% of a total) almost 83% were 28 compounds, in which the prevailing was bornyl acetate-11.35%.

P-052: Acetaldehyde is one of the most potent groups of Toxins

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According to International Agency for Research on Cancer, acetaldehyde is one of most potent and dangerous groups of toxins worldwide and is a carcinogen substance indicating sufficient evidence of carcinogenicity in humans. There is sufficient evidence for the carcinogenicity of acetaldehyde in experimental animals. This chemical is a small highly reactive compound that occurs naturally in various plants, ripe fruits, and vegetables. Many carcinogens, but not all, damage DNA and so generate mutations in genome. For examples, the fungal toxin aflatoxin B1 has been changed to a reactive form by metabolic processes. The reactive form induces bulky aflatoxin-DNA adducts by reacting with guanine in DNA and causes mutations relating cancer.

Here we propose that acetaldehyde is also one of DNA damaging agent. The acetaldehyde forms a guanine-guanine intrastrand crosslink in DNA. The structure of this DNA lesion resembles with bulky UV-induced DNA lesions (e.g. CPD and 6-4pp), leading mutations and cancer. It is likely to be repaired by human nucleotide excision repair preventing cancer and aging. Ethanol of yeast products has been changed to a reactive form acetaldehyde by metabolic processes.
Thus, acetaldehyde from yeast toxin ethanol, like aflatoxin B1, might be thought to be one of environmental genomic toxin.

P-053: A new database on animal toxin-target interactions

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Animal protein toxins have numerous applications in pharmacology, neurology, hematology, and drug research, and are of great interest to a wide range of scientists. Here we describe our current work on the development of a new database that will provide scientists with access to activity profiles and targets (such as channels and receptors) for toxins and derivatives from multiple taxa, including spiders, cone snails, scorpions, sea anemones and snakes. Toxic compound information

will consist of sequence, PTMs, unnatural chemical modifications, name, taxon, and links to UniProtKB, whereas target information will consist of name, gene name, target family, taxon, links to UniProtKB model target and to experimental target. Experimental information will include conditions (types of experiment, target activation, tested cells) and results (activity (yes/no), activity type (agonist/antagonist/potentiator), activity measures (IC(50), % of activity, Kd, and Ki)), and data source (PubMed). While still under development this database is already highly populated with about 1,000 toxic compounds, 250 model targets (mostly human, and some Drosophila), and 4,000 interactions between toxic compounds and their targets. What information would you like to see in this database? We look forward to your input.

P-054: The genotoxic and cytotoxic effects of ochratoxin A and T-2 toxin in rats bone marrow and blood cells

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Mycotoxins are deleterious secondary metabolites of microfungi that contaminate food worldwide. Determining the potential genotoxic and cytotoxic effects of mycotoxins is of great importance for the estimation and prevention of their damaging effects in humans, animals, and crops. Ochratoxin A (OTA) and T-2 toxin (T-2) belonging to the most abundant and harmful mycotoxins, can induce inhibition of DNA and RNA synthesis and oxidative stress in different tissues. Here we studied genotoxic and cytotoxic effects of OTA and T-2 in rat bone marrow and blood leukocytes.

Rats were orally administered OTA or T-2 (25 μg/kg b.w./day) for 21 consecutive days. Bone marrow was flushed out with RPMI 1640 medium from rat femurs using a syringe, venous blood was collected in heparinized tubes. All samples have been studied in triplicates and used for analysis within 15-30 min after collection. Genotoxic effects were studied using single cell gel electrophoresis (comet) assay, % DNA in comet tail was used as the main parameter of genotoxicity according to OECD guideline. Cell viability was measured using trypan blue exclusion test.

OTA and T-2 demonstrated cytotoxic activity both in bone marrow and in blood leukocytes. OTA increased % DNA in the tail in bone marrow and blood leukocytes, while genotoxicity of T-2 was detected only in bone marrow cells. Previously it was shown that OTA may persist in the blood, since OTA binds very strongly to human serum albumin, from where actively can be transported into the cells, while T-2 is actively excreted from the organism. The manifestation of the effect of T-2 in bone marrow can be caused by its belonging to radiomimetic compounds; it is known that bone marrow is the main target tissue of this group of substances.

Thus our data suggest that both mycotoxins are genotoxic and cytotoxic for rats but exhibit the tissue-dependent effect.

P-055: Neurotoxicity and epigenetic effects of ochratoxin A *in vitro*

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Occurrence of mycotoxins in food and environment has been evidenced worldwide and is considered as a significant economical and health problem. Recently, it was reported that mycotoxins cause neuropsychological impairment or mental and emotional disorders but the mechanism of neurotoxicity of certain mycotoxins remains unknown. The correct model for risk assessment of mycotoxins' neurotoxicity is not clearly identified, that hinder the comprehensive characterization of mycotoxins' hazard to humans. In this work, we aimed to study the cyto- and genotoxicity, oxidative stress, and epigenetic changes, as well as reversible/irreversible neurotoxic effects of ochratoxin A (OTA) in human and mouse neuronal cells.

The human SH-SY5Y and mouse HT22 cell lines were selected as test-models. The cytotoxicity of OTA was assessed using calcein-AM/propidium iodide double staining; the genotoxicity of OTA was tested using CBMN assay. The DHE and FPG-comet assays were used to study the OTA induced oxidative stress. The epigenetic effect of OTA was investigated using methylation sensitive comet assay.

It was shown, that OTA is not cytotoxic at the concentration range of 2.5-30 μM in both cell lines. The genotoxic activity was revealed only in HT22 cells at the highest tested concentrations (15 and 30 μM). At the concentrations of 2.5-10 μM OTA induces epigenetic changes in HT22 cells, reflected by the increased level (up to 45%) of unmethylated CpG islands in DNA. The increased level of reactive oxygen species and oxidized purines were detected. All observed processes were reversible after single-dose treatment, but can be retained in a case of chronic exposure. OTA-induced epigenetic changes were not revealed in SH-SY5Y cells, but the low level and reversible oxidative stress was observed after OTA treatment. So, human and animal neuronal cells have different sensitivity against mycotoxin-induced toxicity and careful data extrapolation should be performed when use only animal data.

P-056: New Kunitz-type HCRG peptides of sea anemone *Heteractis cris*

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Marine coelenterates, sea anemones, are a rich source of biologically active peptides. Apart from peptide toxins modulating Kv, Nav, ASICs channels and α-pore-forming toxins, they produce inhibitors of serine proteinases of Kunitz-type, BPTI-Kunitz family. Recently discovered pharmacological potential of representatives of Kunitz-type peptides produced by the species *Heteractis crispa* (Tabakmakher et al., 2015; Gladkikh et al., 2015; Sintsova et al., 2015; 2017) is conditioned by the phenomenon, typical for poisonous organisms, namely, by existence of the multigene families encoding Kunitz-type peptides. This leads to their molecular diversity and functional diversification aimed at expanding of the biological targets range of sea anemones preys and predators. These processes are caused by ancestral genes duplication and paralogs sub- and/or neofunctionalization resulted in Kunitz peptides acquisition of polyfunctionality, subtype-selectivity, and an appearance of analgesic, anti-inflammatory, and anti-histamine activities. Earlier multigene

HCGS family coding more than three dozen of *H. crispa* Kunitz-type HCGS peptides was revealed (Isaeva et al., 2012).

In this work HCRG peptides (33 amino acid sequences of which were derived from cDNA ones) forming distinct HCRG subfamily within HCGS family were found. These highly similar peptides and three native ones (Gladkikh et al., 2012; 2015) form a combinatorial library characterized by point mutations of amino acid residues (observed at both main and weak contact sites as well as along the entire length of the amino acid sequences) which are responsible for: (i) Kunitz homologous' capability to canonical or alternative interaction with a wide spectrum of serine proteinases; (ii) modulation of some subtypes of Kv channels and/or TRP receptors functional activity. Kunitz peptide residues functionally significant for interaction with the biological targets were predicted by molecular dynamic simulations.

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P-057: Oregano ordinary (*Origani vulgaris*) as a source of β-caryophyllene

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Origanum vulgaris belonging to the Lamiaceae family, which is widely spread in flora of Armenia. Our aim is to investigate the influence of the external environment on the accumulation dynamics of the essential oil of Oregano. Essential oil was extracted from fresh plant material by hydro-distillation, using a Clevenger-type apparatus and lasted 3 hours. The essential oil composition was defined by the gas chromatography method. The analysis was carried out using Bruker gas chromatograph, fitted with 60 m × 0.25 mm × 0.25 μm OPTIMA-FFAP column .The results of the gas chromatography analysis showed that in the period of pre-blossoming the basic components of the essential oil of Origanumvulgare are: β-Caryophyllene (7%), α-Cadinol (6,9%), Ent-Spathulenol (6,75%), trans-β-Ocimene (6,13%), D-Germacrene (5,82%), β-Caryophyllene epoxide (5,6%) etc., in the period of blossoming the basic components of the essential oil are:β-

Caryophyllene epoxide (13,36%), β-Caryophyllene (8,18%), o-Cymene, (5,22%), Germacrene D (3,80%), trans-β-Ocimene (3,81%) etc., in the period of fruiting the gas chromatography results showed the basic components of the essential oil of O. vulgare: β-Caryophyllene epoxide (11,2%), o-Cymene (9,41%), D-Germacrene (9,22%), α-Cadinol (6,93%), β-Caryophyllene (6,41%), β-Ocimene (5,97%). O.vulgare in flora of Armenia belongs to the fourth chemotype andthe main component of essential oil of O. vulgare is β-Caryophyllene.The quantitative and qualitative composition of essential oil is vitally different and changes in the vegetation periods.

P-058: Evaluation of the structure and function of recombinant disintegrins with antiplatelet activity from *Bothrops jararaca* venom

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Disintegrins are a family of small peptides that modulate integrin receptors, which play a fundamental role in thrombosis, leading cause of death, accounting for 1 in 4 deaths worldwide. Our group studies two RGD disintegrins from *Bothrops jararaca* snake, jarastatin (JAST), isolated from its venom with potent antiplatelet activity and jararin (JARR), a novel disintegrin identified from the cDNAs snake gland analysis. These disintegrins may possess different selectivity for integrins, due to differences within the motif sequence. That motivated us to characterize these disintegrins as models for the study of prototypes of new antiplatelet drugs. The aim of this work was to produce the recombinant forms and to characterize them in regard to their structure and hemostasis effect. rJARR was fused with Thioredoxin and the expression was performed in *Escherichia coli*. The disintegrin was purified using affinity chromatography, cleaved by enterokinase, followed by reverse phase chromatography. rJAST expression was performed in *Pichia pastoris*. The disintegrin

was purified by size-exclusion chromatography. The identity and integrity of these disintegrins were assessed by ESI-Q-ToF mass spectrometry (MS) and one-dimensional NMR spectra. The recombinant proteins were evaluated for their inhibitory activities on platelet aggregation induced by ADP. rJARR and rJAST expression resulted in a yield of 2.3 mg/L and 4 mg/100 mL respectively. The experimentally MS value of rJARR was 7908.6 Da and rJAST was 7752 Da. The NMR analysis showed rJARR to be partially folded, while rJAST showed to be well folded. The aggregation assay showed that rJARR and rJAST inhibit ADP-induced platelet aggregation with an IC_{50} of 550 nM, and 281 nM, respectively. rJAST showed similar activity to the native form. In this regard, we succeed to express and characterize rJARR and rJAST. The elucidation of their structure will contribute to the understanding of its structure/activity relationship.

P-059: THE ANALYSIS OF CHEMICAL COMPOSITION OF Smoke Trees Leaves (CotinuscoggygriaScop.), GROWING IN ARMENIAN FLORA BY GC-MS METHOD

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The Armenian flora is stood out by the variety of its medicinal and edible plants. Based on the world literature data, climatic-geographical factors have a great influence on chemical composition of plants, especially on volatile oil's chemical composition. In this point of view it is interesting the investigation of the chemical composition of the volatile oil of Smoke trees leaves growing in Armenian flora. The object of our study was Smoke tree's leaves collected from Yerevan.The harvesting and drying process were carried out by WHO GACP instructions. Volatile oil was derived by hydro-distillation method and the chemical composition of volatile oils was determined by GC-MS method.Volatile oil analysis was carried out by the gas chromatography- mass spectrometry method.An OPTIMA-FFAP capillary column was used for separation of volatile oil compounds. In the volatile oil of Smoke tree leaves 24 compounds were determined, from which 10

were of terpenoid structure. Almost all terpenoids were monocyclic monoterpenoids. Acyclic monoterpen linalool also was present (3.77 %). The predominated monocyclic monoterpen was pulegone (25.49%). From the monocyclic monoterpenoidscys-cinerolone (21.81%), cys-menthone (13.30%), α- terpineol (12.94%), eucalyptol (9.04%), L-4-terpineol (8.20%), 1,4-dihydroxy-para menth-2-ene (3.72%) were present.First time the chemical composition of volatile oil of several plants of Armenian flora such us Smoke tree`s leaves were prevailed monoterpenpulegone and the monocyclic monoterpenoidscys-cinerolone .

P-060: Enzymatic activity of jellyfish venom and treatment for jellyfish sting

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Jellyfish *Nemopilema nomurai* is a very dangerous animal because of its strong toxicity. It often bloomed in the coast of China in recent years and caused thousands of people stung and even resulted in deaths every year. Jellyfish venom is the primary cause of sting. A study on jellyfish venom is the key way to treatment jellyfish stings. We analyzed the components of venom from jellyfish N. nomurai by venomics and venom gland transcriptomics, 218 toxins were identified, including 47 phospholipase A2s and 33 metalloproteases. Enzymatic properties of jellyfish N. nomurai nematocyst venom were analysed. *N. nomurai* nematocyst venom exhibited various enzymatic activities, of which metalloproteinases activity and PLA2s-like activity were predominant. Moreover, the enzymatic activities of metalloproteinases and PLA2s-like were dependent on different physiochemical conditions such as temperature, pHand divalent ions. Hemolysis is commonly used as an index for jellyfish stings. Metalloproteinase inhibitors EDTA and batimastat inhibited the hemolysis and the metalloprotease activity. Varespladib, as sPLA2 selective inhibitior, inhibited the hemolysisand the MIC was 1.6 μM. So, we speculated that enzymatic activity played

an important role in the jellyfish stings. In addition, we developed an external drug JSM for treatment jellyfish stings according to the enzymatic activity and physical and chemical properties of jellyfishvenom. JSM is deep yellow and pH of JSM is 6.5. More than 900 voluntaries were involved to evaluate the effect of JSM on jellyfish stings from 2012 to 2017. JSM can shorten thecourse of dermatitis and visibly relieve the itching, pain and swelling according to the dataof preliminary investigation.

P-061: Standardization of the raw material Ziziphora linopodioides lam. by HPLC and spectrophotometric methods

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The aim of the current study was the qualitative and quantitative analysis of the extracts obtained from the raw material Z.clinopodioides by HPLC and spectrophotometry methods. With HPLC analysis was confirmed the qualitative phenolic compositions of the substances previously identified by the thin layer chromatography.

The modified spectrophotometric absorption method was used to quantify the sum of flavonoids in the raw material Z.clinopodioides. This study is the first report of definitionthe specific absorption coefficient for the flavonoid 7-methyl sudahitin$E_{1cм}^{1\%} = 920$ which was included in the modifying formula. The totalamount of flavonoids in dry raw material in accordancewith 7-methyl sudahitin could be calculated by the formula:

$$x = \frac{D_{x.} \cdot 250 \cdot 50}{920 \cdot 2m}$$

m –themass of the sample, D_x-an optical density of the test solution atλ = 207nm; 250 - a solution volume, (ml); 50 - the aliquots volume was taken from solution (ml); 920 - the specific absorption coefficient flavonoid 7-methyl sudahitin.

HPLC analysis showed the presence of two phenolic compounds: flavonoid apigenin, and phenyl - propanoidverbascoside.The apigenincontent in various samples of Z.clinopodioides-ranged from0,0024to0,01 mg/ml, and the verbascoside- ranged from 0,114 to0,504 mg/ml.

It had been shown that the plant Z.clinopodioides accumulated the significant quantity flavonoids - total amount from 2,57 up to 4,18%.

The current study indicated that the raw material wild Z.clinopodioides, cultivated in soil and in hydroponics conditions was the source of flavonoids, and the quantity of which vary not only environmental but also cultivated conditions. For the first time in ethanol extract from Z.clinopodioideswas determined the presence of two phenolic compounds; the flavonoid apigenin, and phenyl - propanoidverbascoside, which could be used as a biomarker for standardization the raw material Z.clinopodioides growing in the floras of Armenia and Artsakh.

P-062: Comparative study of protein profile and enzymatic activities of venoms from snake species that compose the *Bothrops neuwiedi* complex

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The snakes that composed the so-called "*B. neuwiedi* complex" underwent a taxonomic revision and as a result of this analysis, it was proposed that this complex is in fact composed by 7 full species: *B. neuwiedi*, *B. diporus*, *B. lutzi*, *B. mattogrossensis*, *B. pauloensis*, *B. pubescens* and *B. marmoratus*. Since the characterization of the venoms of these species after the taxonomic revision

of the group is scarce, the aim of this study is to characterize and compare the protein profile and the enzymatic activities of venoms from six of these species (*B. neuwiedi*, *B. diporus*, *B. marmoratus*, *B. mattogrossensis*, *B. pauloensis* and *B. pubescens*). For comparative purposes, we have included the species *B. erythromelas* in our analyses. All venom samples (6 to 10 of each species) were analyzed individually. Protein profile obtained by 1-DE revealed some species-specific band patterns, but also stressed the intraspecies venom variation, showing differences concerning the intensity and the presence/absence of particular protein bands. Amongst the species analyzed, *B. pauloensis* venom displayed the lowest proteolytic activity upon azocasein and collagen (Azocoll). Conversely, *B. pauloensis* and *B. erythromelas* showed the highest PLA_2 activity upon the synthetic substrate 4-NOBA. In contrast, LAAO activity upon L-methionine was higher in *B. diporus* and *B. pubescens* venoms, while *B. erythromelas* showed a remarkable individual variability regarding the presence/absence of this activity. These preliminary results revealed some particular features of the venoms of the five species under analysis, regarding protein composition and enzymatic activities. However, the characterization of the protein profile of these venoms by HPLC and proteomic analysis by mass spectrometry, besides the comparison of their pathophysiological activities, will complement the characterization of these venoms.

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P-063: The comparision of *Montivipera raddei* and *Macrovipera lebetina obtuse* viper venoms effects on human erytrocite membrane ATPase activites.

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Armenia is a country with rich herpetofauna. Many species of snakes in our country are very endemic and peculiarities of its toxicology are still unknown. *Macrovipera lebetina obtuse* (MLO) and *Montivipera raddei* (MR) represent the most important poisonous snakes in Armenia. The studying of snake venom's influence on organism is a vital question today for Armenia both in case of safety of population and in aspect of using of snake toxins in pharmacology. The present study was in part prompted by an interest in the changes of the condition of native human red cell

membranes under the influence of MLO and MR venoms. We have studied influence of venom on the erythrocyte ghosts by fluorescent microscopy (AmScope, USA). Eerythrocyte membranes were visualized with ANS fluorescent probe. The erythrocyte ghosts were deformed after adding the venoms. We have shown that in presence MR venom ghosts were shrinking during 58 seconds. In presence MLO venom duration of shrinking was increase; they shrink within 3 min, and pull in. We also studied activities of Na^+/K^+ ATPase and Ca^{2+}-activated Mg^{2+}-dependent ATPase in the absence and in the presence MR and MLO venoms. Venoms was added into the assay mixture with low, sub-lethal (0.35 mg/kg approx. 0.5 LD 50 for rat) and lethal concentrations in accordance with LD50. It was shown that Na^+/K^+ ATPase activity in erythrocytes membranes was increased in the presence of the MLO venom (low concentration ~1.81 times, sub-lethal concentration ~3.83 times and lethal concentrations ~4.28 times respectively). In comparison with MLO, the addition of MR venom into assay mixture increased Na^+/K^+ ATPase activity stronger (low concentaraton ~5.28 times, sub – lethal concentration ~4.5 times and lethal concentrations ~2.93 times respectively). Under these conditions Ca^{2+} ATPase activity was decreased (low concentration ~3.37 times, sub-lethal and lethal concentrations ~17.93 times respectively) in presence MLO venom and increased at low concentaration ~1.59 times, then decreased at sub–lethal concentration ~2.87 and lethal concentrations ~4.41 times respectively in presence MR venom. These results suggest that ATPase activity is very sensitive to venom components and venoms influence leads to possible conformation changes in ATPases.

P-064: The genome and transcriptome of the Indian Cobra

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Najanaja, the India cobra, is one of the "big four" snakes in India that is responsible for the over 50,000 deaths associated with snake bites annually in India. A majority of the anti-venom available for treating snake bites are developed from immunized horses. While the anti-venom provides a degree of relief, it has side effects and is not always effective. The anti-venom technology used today dates back to 1895 and has not adequately evolved to fully leverage the advances in genomics and molecular biology.

Next generation sequencing technologies have enabled rapid and cost effective genome and transcriptome analysis. Such information can be used to develop humanized synthetic anti-venoms

that are effective with minimal to no side-effects. With this in mind we have sequenced the genome of the *Najanaja*. We generated ~60 Gb of Oxford Nanopore and ~40 Gb of PacBio single molecule long read data, and ~120 Gb of short read Illumina data. Also, we generated ~300 Gb of BioNano optical mapping data. We combined these data to produce a 2.1 Gbp assembly that is highly contiguous, comprised of only 351 scaffolds and an N50 of 106.9 Mb. We were able to identify 84% of the core genes in our assembly.

In addition to the genome, we sequenced RNA from nine different tissues. In a particular, we sequenced RNA from the venom gland using PacBio technology to obtain full-length transcripts. Starting with 484,742 reads, we derived 211,786 non-chimeric reads. Applying iterative clustering and error correction (SMART Link 4.0 analysis) we obtained 61,788 unique reads. Of these 50,482 transcripts had a match to a known protein sequence. In total we identified 9,372 unique proteins with a homolog. Among the top proteins represented in the venom gland transcriptome were glutathione peroxidase 3, protein disulfide-isomerase, cobra venom factor, pyruvate kinase, creatine kinase M-type, four and a half LIM domains protein 1, cytotoxin 5, multiple neurotoxins and cardiotoxins.

Together, these data can be used to better understand the biology and evolution of the India cobra and also aid in the development of effective anti-venom using synthetic biology techniques.

P-065: Morphological and functional alteration of human erythrocytes caused by some Iranian vipers' venom

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Snake bites are an endemic public health problem in Iran, both in the rural and urban area. Viper venom as a hemolytic biochemical “cocktail” of toxins, primarily cause to the systemic alteration of blood cells. In the sixties and seventies, erythrocytes or red blood cells (RBCs) were extensively studied, but the mechanical and chemical stresses commonly exerted on RBCs continue to attract interest for the study of membrane structure and function. Here, we monitor the effect of Vipera latifi, Macrovipera lebetina obtusa and Montivipera raddei venom on human erythrocytes ghost membranes using phase contrast and fluorescent microscopy and changes in ATPase activity

under snake venom influence in vitro. The ion pumps [Na+, K+]-ATPase and [Ca2+ + Mg2+]-ATPase plays a pivotal role in the active transport of certain solutes and maintenance of intracellular electrolyte homeostasis. We also describe the interaction of these venoms with giant unilamellar vesicles (GUVs) composed of the native phospholipid mixtures visualized by the membrane fluorescence probe, ANS, used to assess the state of the membrane and specifically mark the phospholipid domains. To confirm molecular recognition of a collagen receptor on human erythrocytes by disintegrins from viper venoms, a surface acoustic wave-biosensor was applied. The data provide evidence for a direct confirmation of disintegrin binding to erythrocyte ghost membrane and thus, contribute to prove the presence of integrins in the red cell membranes earlier neglected. Therefore, disintegrins are coming into light as attractive pharmacological tools for suggesting novel approaches to the generation of red blood cells aggregation inhibitors.

P-066: Application of bee venom on immunological and neurological diseases

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The western honey bee or European honey bee (*Apis Mellifera*) is the most common of the 7–12 species of honey bee worldwide. The genus name *Apis* is Latin for bee, and *Mellifera* is the Latin for honey-bearing, referring to the species' production of honey for the winter. The western honey bee was one of the first domesticated insects, and it is the primary species maintained by beekeepers to this day for both its honey production and pollination activities. The honey bee is an important pollinator of crops and this service accounts for much of the species' commercial value. A primary product of honey bees is honey. Honey is the complex substance made from nectar and sweet deposits from plants and trees which are gathered, modified and stored in the comb by honey

bees. Mature worker bees secrete beeswax from glands on their abdomen, using it to form the walls and caps of the comb. Bees collect pollen in a pollen basket and carry it back to the hive, where after undergoing fermentation and turning into bee bread becomes a protein source for brood-rearing and excess pollen can be collected from the hive. Bee brood, the eggs, larvae, or pupae of honey bees, is edible and highly nutritious. Propolis is a resinous mixture collected by honey bees from tree buds, sap flows or other botanical sources, which is used as a sealant for unwanted open spaces in the hive. Royal jelly is a honey-bee secretion used to nourish the larvae and queen. It is marketed for its alleged but unsupported claims of health benefits. Honey bee venom has long been used in Korea to relieve pain symptoms and to treat inflammatory diseases, such as rheumatoid arthritis. It contains a number of very volatile compounds which are easily lost during collection; it is considered a rich source of enzymes, peptides and biogenic amines.

P-067: Proteomics, transcriptomics and toxicity analysis of venom proteins from jellyfish *Cyanea nozakii*

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Jellyfish sting became a worldwide issue of critical concern to human health and safety in coastal areas in recent decades. Cyanea nozakii is one of the three dominant species bloomed in China Sea. Every year, hundreds of thousands of people got stung and those victims suffered itch, edema, myalgia, dyspnea or shock. However, it is still unclear how many and what types of toxins are in the venom. We combined transcriptomics and proteomics approach to investigate the venom composition of jellyfish C. nozakii. A total of 174 potential toxin proteins were identified, including 27 proteins homology to the toxins from venomous animals, including phospholipase A2, zinc metalloproteinase-disintegrin agkistin, serine protease inhibitor, plancitoxin-1, alpha-

latrocrustotoxin-Lt1a, etc. Toxicity analysis showed that cardiotoxicity of the venom should be responsible for the death caused by this jellyfish venom. Our findings not only provide a comprehensive understanding the composition and toxicity of the venom from C. nozakii, but also will be very helpful for the development of effective treatments for jellyfish sting in the future.

P-068: Antivenom against *Vipera berus* is effective against *Macrovipera lebetina*

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To date there is no drug to treat *M.lebetina*envenoming in Russian Federation. The drug "Antivenom against *V.berus*", registered in the Russian Federation was analyzed.This antivenom was shown to neutralize lethal activity (4LD50) of both *V. berus* and *M. lebetina* venom with equal potency. The effective neutralizing dose was found to be 2 mg of venom per ml of the antivenom.

At first, the LD50 values for the venoms of *V.berus* and *M.lebetina* were determined. For this reason, 5 groups of 5 mice (20 g) each were taken. The venom was injected intraperitoneally with 500 µl in the following doses: 0.5, 1, 1.5, 2 and 4mg/kg. The mortality was recorded for 2 days. LD50 for *V.berus* was 1.08 mg/kg, and for *M.lebetina* 3.2 mg/kg.

To determine the neutralizing potency of the antivenom against each venom 4 groups of 5 mice were taken. Each mouse was injected intraperitoneally with 500 µl of the mixture containing *V.berus* or *M. lebetina* venom (4LD50) and antivenomin the following doses: 2, 4, 6, 16 mg of venom per ml of the antivenom. All mixtures were prewarmed for 30 minutes at 37°C in the water bath prior to administration. Mortality was recorded in two days. The neutralizing potency of the antivenom was evaluated according to Probit analysis. Neutralizing activity of the antivenom against both venoms was estimated to be 2 mg/ml.

There are 2 main venomous snakes in the Russia: *V. berus* and *M. lebetina* (Dagestan republic). However, the antivenom against the *M.lebetina* envenoming is currently absent. The data obtained could be used for further medical application extension of "Antivenom against *V.berus*",

registered in the Russian Federation.It is interesting, thatalthough snakes *M.vipera* and *V. berus* belong to different genera diversified 20 million years ago; their venoms have an immunological similarity.