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COMPARATIVE STUDY OF FIVE COBRAS' VENOMS ANALGESIC CAPACITY

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Abstract

Every day, a lot of people suffer from different types and intensity pain. Pain is probably the most common symptomatic reason to seek medical consultation. Unfortunately, despite improved knowledge of underlying mechanisms and better treatments, many people who have any pain receive inadequate care and non-effective drugs. Although the pain transmission channels are intensively studied, and the drug market is constantly replenished with new analgesics, it is well known that existing medications for the treatment of pain are often associated with serious side effects and rapid development of tolerance (moderate efficiency, physical dependence, respiratory arrest, suffocation, cardiac arrest, etc.). Thus, there is a need for new, more effective remedies. For this reason, despite the presence of a large number of anti-pain drugs, research and development of more effective and safe means for anaesthesia continue.

Natural resources, particularly venoms, are a perspective supplier of antinociceptive and anti-inflammatory medicines. Venoms are complex mixtures of bioactive substances with high selectivity for physiological processes, including modulation of different ion channels, receptors function, and metabolic pathways. Thus, venoms represent an extensive source of molecules for the development of therapeutic agents.

The goal of this study was the comparison of antinociceptive effects of five different cobras' venom antinociceptive action of cobras' venoms was carried out under the same experimental conditions, at the same doses, during acute and inflammatory pain in mice in "formalin test". To avoid toxic effects, the sublethal dose of each cobra venom (approximately 1/10 LD 50) was selected.

The behavioural study showed that all tested venoms had a slight sensitizing effect in the acute phase during the first 5 min. In the second, inflammatory phase (16*25min), all tested cobra's venoms (3μg/0.1ml, intraperitoneal) showed significant antinociceptive action, particularly the Naja naja oxiana venom decreased pain sensitivity by 48.4%, the Naja naja pallida venom by 75.4%, the Naja naja nigricincta venom by 38.5%, the Naja naja kaouthia venom by 33.2%, and the Ophiophagus

Hannah venom by 78.3%, ($p<0.05$). The analgesic capacity of Analgin and Diclofenac under the same conditions were 77.9% and 88.7%, respectively. Thus, the Naja n. pallida and Ophiophagus Hannah venoms have shown the most expressed antinociceptive action, and they have competitive effectiveness compared to classic analgesics. They may be chosen as the most effective from tested venoms for further development of pain relief remedies.

Keywords and phrases: pain, antinociception, cobra venom, formalin test.

ՀԻՆԳ ԿՈԲՐԱՆԵՐԻ ԹՈՒՅՆԵՐԻ ՑԱՎԱԶՐԿՈՂ ԱԶԴԵՑՈՒԹՅՈՒՆՆԵՐԻ ՀԱՄԵՄԱՏԱԿԱՆ ՀԵՏԱԶՈՏՈՒԹՅՈՒՆ

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Համառոտագիր

Ամեն օր բազմաթիվ մարդիկ տառապում են տարբեր բնույթի ցավերից: Ցավն այն առավել հաճախ հանդիպող ախտանշանն է, որի պատճառով հիվանդները դիմում են բժշկին: Չնայած ցավային ազդակների փոխանցման ուղիներն ու մեխանիզմները մշտապես ուսումնասիրվում են և դեղերի շուկան անընդհատ համալրվում է նոր ցավազրկողներով, սակայն հայտնի է, որ դրանք հաճախ ունենում են անցանկալի կողմնակի հետևանքներ (չափավոր արդյունավետություն, ֆիզիկական կախվածության առաջացում, շնչառության կանգ, շնչահեղձություն, սրտի կանգ և այլն): Ուստի՝ նոր, ավելի արդյունավետ դեղերի հայտնաբերումը արդիական խնդիր է:

Բնական ռեսուրսները, մասնավորապես՝ կենդանական թույները, համարվում են հակացավային և հակաբորբոքային միջոցների հեռանկարային հումք: Թույները կենսաբանորեն ակտիվ միացությունների բարդ խառնուրդներ են, որոնք ունեն բարձր խնամակցություն տարբեր կենսաբանական գործընթացներում:

Սույն հետազոտության նպատակն է համեմատել հինգ տարբեր կոբրաների թույների հակացավային ազդեցությունները: Հետազոտությունները իրականացվել են միևնույն փորձարարական պայմաններում, ֆորմալինային թեստի սուր և բորբոքային փուլերի ընթացքում:

Հետազոտությունները ցույց են տվել, որ հետազոտված բոլոր թույները ֆորմալինային թեստի առաջին փուլի (0-5րոպե) ընթացքում ցուցաբերել են թույլ սենսիտիզացնող ազդեցություն, իսկ երկրորդ՝ բորբոքային փուլում ցուցաբերել են նշանակալի ցավազրկող ազդեցություն: Մասնավորապես, NNO-ի թույնը ճնշել է ցավազգայնությունը 48.4%-ով, NNP-ի թույնը՝ 75.4%-ով, NNN-ի թույնը՝ 38.5%-ով, NNK-ի թույնը՝ 33.2%-ով և OH-ի թունը՝ 78.3%ով ($p<0.05$): Սա այն դեպքում, երբ անալգինի ցավազրկող ունակությունը միևնույն պայմաններում 77,9% է, իսկ դիկլոֆենակինը՝ 88,7%: Ուստի՝ NNP և OH թույների ցավազրկող ազդեցությունները մրցունակ են ստանդարտ ցավազրկողների հետ և կարող են կիրառվել հետագայում՝ նոր ցավազրկող միջոցների մշակման համար:

Բանալի բառեր և բառակապակցություններ. ցավ, հականոցիցեպցիա, կոբրայի թույն, ֆորմալինային թեստ:

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ АНАЛЬГЕТИЧЕСКОЙ СПОСОБНОСТИ ЯДОВ ПЯТИ ВИДОВ КОБР

ЛИЛЯ ПАРСЕГЯН

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Аннотация

Каждый день множество людей страдает от болей различных типов и интенсивности. Боль, вероятно, является наиболее частой симптоматической причиной обращения за консультацией к врачу. Хотя пути и механизмы передачи боли интенсивно изучаются, а рынок лекарств постоянно пополняется новыми анальгетиками, многие люди, страдающие от боли любого типа, получают неадекватную помощь и неэффективные лекарства, с другой стороны, хорошо известно, что действие существующих лекарств для лечения боли часто связано с серьезными побочными эффектами и быстрым развитием толерантности (умеренная эффективность, физическая зависимость, остановка дыхания, удушье, остановка сердца и т.д.). Таким образом, поиск новых, более эффективных лекарств является актуальной задачей.

В этом плане животные яды являются перспективным ресурсом для разработки антиноцицептивных и противовоспалительных лекарственных средств. Яды представляют собой сложные смеси биоактивных веществ, которые обладают высокой селективностью в отношении физиологических процессов, включая

модуляцию различных ионных каналов, функциональных рецепторов и метаболических путей.

Целью нашего исследования было сравнительное изучение противоболевого действия ядов пяти различных видов кобр. Антиноцицептивное действие ядов кобр изучали в одинаковых экспериментальных условиях при острой и воспалительной боли у мышей в «формалиновом тесте». Были выбраны сублетальные дозы ядов кобр (примерно 1/10 LD 50), что исключало их токсическое действие.

Исследование показало, что все испытанные яды имели небольшой сенсибилизирующий эффект в острой фазе в «формалиновом тесте». Во второй, воспалительной фазе (16*25мин.), все протестированные яды (3мкг/0.1мл, внутрибрюшинно) показали значительное антиноцицептивное действие, в частности, яд NNO снижает болевую чувствительность на 48.4%, яд NNP - на 78.3%, яд NNN - на 38.5%, яд NNK - на 33.2% и яда OH - на 78.3% ($p < 0,05$). И это в том случае, когда анальгетическая способность анальгина в тех же условиях составляет 77,9%, а диклофенака - 88,7%. Таким образом, яды NNP и OH обладают конкурентоспособной эффективностью по сравнению с классическими анальгетиками и могут быть выбраны как наиболее эффективные из испытанных ядов для дальнейшей разработки обезболивающих средств.

Ключевые слова и словосочетания: боль, антиноцицепция, яд кобры, формалиновый тест.

Introduction

The objective definition of pain is endorsed by the International Association for the Study of Pain: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" **[1].**

Pain is a protective but unpleasant sensation, which occurs in response to injury, disease, or cellular damage. Pain can be classified according to its duration (acute or chronic), its underlying cause (nociceptive or neuropathic) and its severity (mild, moderate or severe).

Nociception is the detection of painful stimuli resulting from heat, cold, trauma or inflammatory mediators, which are then transmitted to the CNS. Nociceptive pain results from stimulation of nociceptors on peripheral nerve terminals, often by signalling molecules released from damaged tissue. This nociceptive pain serves a protective purpose in alerting the body to tissue damage and preventing further damage due to continued use or exposure to harmful conditions. Neuropathic or nerve pain results from lesions or dysfunction in the nervous system. It can be peripheral or central in origin and is often caused by brain or spinal cord lesions. Here, we review the nociceptive aspect of pain perception.

Pain is a symptom of different disorders and conditions. Therefore, in the last decades, it has been the subject of several types of researches. Unfortunately, not all of the mechanisms of pain signalling are yet very well understood. The type of pain, and consequently its treatment, may vary, and there are still a lot of difficulties, particularly for veterinarians, for example, to prescribe the best treatment or even recognize the different patterns of behaviour that may indicate that the animal is suffering from pain **[2]**.

Three groups of drugs widely used in clinical practice (excluding local anaesthetics) have a direct analgesic effect: steroidal anti-inflammatory drugs, opioid analgesics, and non-steroidal anti-inflammatory drugs (NSAIDs).

One of the most common classes of analgesic drugs that are used as potent painkillers is opioids. However, besides causing tolerance, addiction and constipation, the administration of opioids may induce respiratory depression as a severe side effect **[3].**

The use of non-steroidal anti-inflammatory drugs (NSAID) is very effective for the treatment of pain. However, it also may cause some serious side effects, such as irritation of the gastrointestinal tract and contribution to the development of renal function abnormalities **[4]**.

The use of steroid drugs is often undesirable and limited due to their metabolic action on the hormonal status of the organism.

Therefore, there is an urge to discover new preparations that may be used to treat different types of pain without causing the above-mentioned undesirable side effects induced by the use of the currently available drugs. Among many candidates, other venoms can be helpful for the research and development of such preparations.

Therefore, many pharmaceutical companies are interested in the development of drugs based on venoms or their separate components. Many different toxins and derivatives obtained from various groups of animals have been described as able to relieve pain through activation or blockage of different molecular targets **[5].**

Toxins have diverse pharmacology and act on a broad spectrum of targets: ion channels, receptors, enzymes, cell membranes, or key metabolic steps.

To date, the amino acid sequence of more than 2’500 toxins (venom origin) has been determined from only a few hundred species. With a reserve of several a lot of biologically active ingredients, venomous animals constitute one of the richest sources of physiologically active peptides. So, venom components are an increasing source of approved drugs. To date, many medical preparations derived from venom components are present on the market (Captopril, hypertension; Aggrastat/Tirofiban and Integrilin/Eptifibatide, thrombo-embolic events; Prialt, chronic intractable pain; Sinergel remedy for pain in the spine; Naxin as an algesic and anti-Inflammatory drug, et al.), underlining the potential of venom biodiversity as a source of selective molecules for pharmacological targets involved in pain **[6].**

Among snakes, there are the cobrotoxin, isolated from the venom of Naja naja atra and cobratoxin from Naja kaouthia, which present high affinity to different subunits of nicotinic acetylcholine receptors (nAChRs) and exert antinociception **[7].** Venom from spitting elapids (N. atra, N. kaouthia, Naja nigricincta, Naja pallida) contains 67-73% three finger toxin **[8]**, which led to significantly decreased nociceptive sensitivity in rodent pain models of inflammatory and neuropathic pathways by selectively blocking the voltage-gated sodium channel Nav1.8 **[9]**. The neurotoxin hannalgesin, isolated from Ophiophagus Hannah, exert antinociception via activation of the opioid pathway **[10].**

In this study, we have compared the analgesic effects of various types of cobras' venoms on the nociceptive behaviour of mice under the same experimental conditions to identify the venom with the highest analgesic potential. Cobra venoms were selected so that there is a difference in their evolutionary origin. In the present study, the antinociceptive effect of Naja naja oxiana (NNO), Naja n. pallida (NNP), Naja n. nigricincta (NNN), Naja n. kaouthia, and Ophiophagus hannah (OH) venoms was investigated.

Materials and methods

Animals: This study was conducted following "Principles of Laboratory Animal Care" and carried out according to the European Communities Council Directive of September 22 2010 (2010/63/EU). In this study, 20±2g adult albino mice were used, 6-12 mice in each group, totally were investigated, eight groups.

Reagents: Cobra's venoms were milked and dried in the Orbeli Institute of Physiology of National Academy of Sciences of Armenia, in Armenian zoo or purchased from "Sigma-Aldrich", "Merck" "Reanal" and "H. Lundbeck A/S" companies as indicated. As a standard analgesics, Sodium Metamizole (Analgin, Yerevan Chem. Pharm. Factory) and Diclofenac Sodium (Diclofenac, Hemofarm, Serbia Vrsac, N011648/03) were used. Formalin and other chemicals were of analytical or sequencing grade.

Methods: We used the formalin test **[11]** to evaluate the antinociceptive properties of the crude venoms after intraperitoneal (ip) injection. A slight modification of the formalin test appropriate for the testing of mice is described. "Biting/Licking of hind paw" method of estimating nociceptive behaviour in mice was used. 0.02ml 5% formalin was injected intraplantar (IPL) in the hind paw of mice (fig 1). Naja naja oxiana (NNO), Naja naja pallida (NNP), Naja naja nigricincta (NNN), Naja naja kaouthia (NNK), and Ophiophagus Hannah (OH) venoms were injected intraperitoneal (IP, 15 min. before formalin IPL injection), the dose of venoms was 3µg/0.1ml. The quantity of biting/licking of the hind paw was recorded during 45 minutes after formalin IPL injection **[12].**

Statistical analysis: Data analysis was performed by Graph Pad Prism 8 software (Graph Pad Software Inc., USA, 2003). The results of observations at each minute were averaged both for the entire experimental period (45 min) and in 5 min intervals (for the analysis of dynamic changes in nociceptive behaviour). One-way ANOVA followed by Bonferroni Multiple Comparison Test was used for statistical analysis. Values of $p<0.05$ were considered as significant. Results are given as Mean value ± Standard Deviation (Mean ± SD).

Results

First, the formalin test for the intact mice was carried out. 0.02ml of 5% formalin was injected into the hind paw of mice. The nociceptive behaviour was recorded for 45min. Then we used Sodium metamizole (Analgin, 8.3 mg/kg) and Sodium Diclofenac (10mg/kg) as a comparison drug. Dosage selection was based on protocols widely used in the literature **[13]**. The analgesics were injected intraperitoneal (IP, 15 min. before formalin IPL injection). The results are shown in Figures 1 and 2.

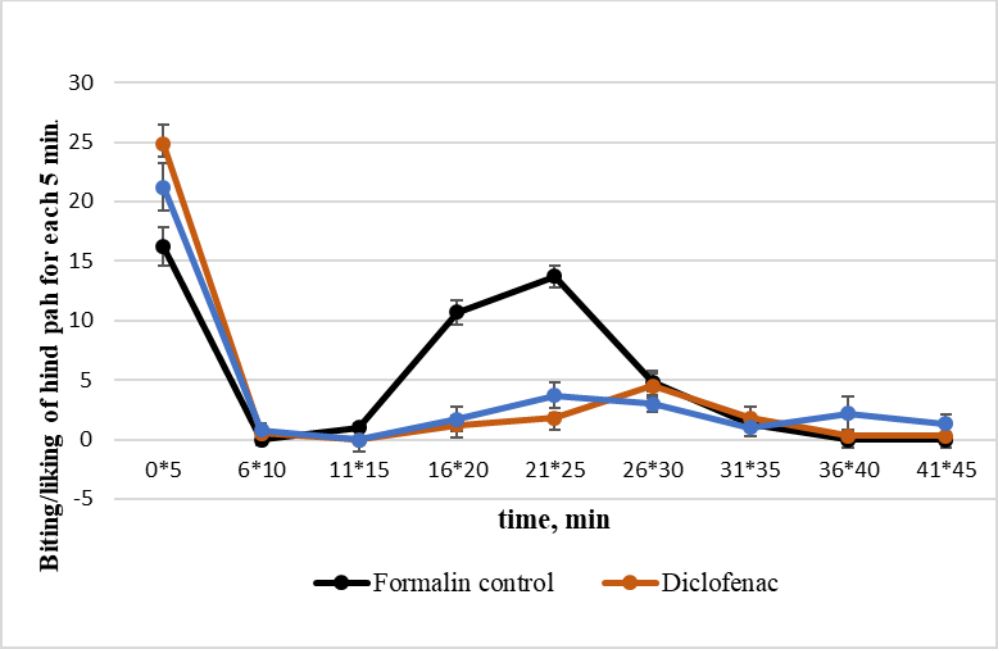

Fig. 1. The analgesic effects of Analgin and Diclofenac vs Formalin

It's known **[14]** that in acute pain phase affects only local anaesthetics. However, Diclofenac and Analgin showed a significant pain relief effect in the second phase (16*25min, $p<0.05$).

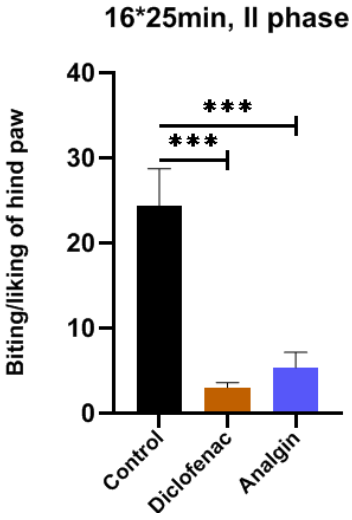

Fig 2. Analgin and Diclofenac significantly decreased pain sensitivity in the II phase of pain development

The analgesic effects of cobra's venoms were compared with this data. The sublethal dose of cobra venom was selected at approximately 1/10 LD_{50} to avoid toxic effects. 3µg in 0.1ml aliquots of NNO, NNP, NNN, NNK, and OH venoms were injected intraperitoneal (IP, 15 min. before formalin IPL injection). The results are shown in figure 3.

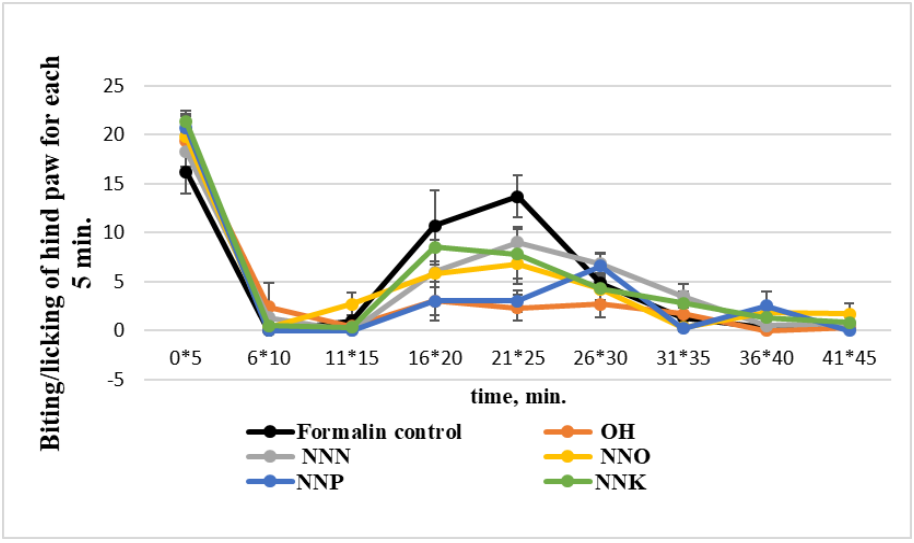

Fig 3. The analgesic effects of cobra venom

It was obtained that all tested venoms in the second stage showed an analgesic effect. The OH and NNP venoms have shown the most expressed antinociceptive action ($P<0.05$) (fig. 4).

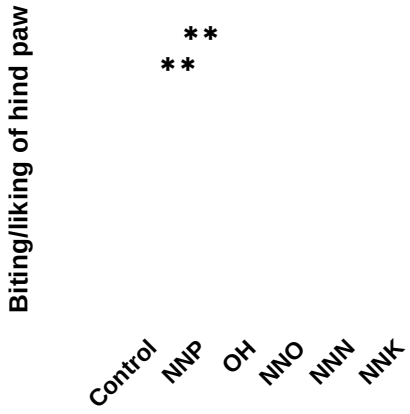

Fig. 4. Antinociceptive action of cobra's venoms in II (16*25min) phase

Comparison of the analgesic capacity of all tested venoms and standard analgesics are shown in table 1 and figure 5.

Table 1. The analgesic effect of cobras' venoms and standard analgesics vs Formalin

Intervals in min	0*5	6*10	11*15	16*20	21*25	26*30	31*35	36*40	41*45
	Mean±SD								
Formalin control	16.2±5.2	0±0	1±1.5	10.7±8.5	13.7±5	4.8±5.3	1.3±2.4	0.3±0.8	0.3±0.8
OH	19.4±7.1	2.4±6.4	0.4±1.1	3±5.2	2.3±3.3	2.7±3.6	1.7±1.5	0±0	0.3±0.3
NNN	18.3±4.8	1.3±3.3	0±0	6±7.6	9±3.5	6.8±2.7	3.5±2.8	0.5±1.2	0.7±1.6
NNO	19.8±3.4	0.3±0.8	2.7±2.9	5.8±6.6	6.8±5.1	4.2±3.8	0.3±0.8	1.8±1.7	1.7±2.7
NNP	20.7±2.9	0±0	0±0	3±3.3	3±2.8	6.5±2.9	0.2±0.4	2.5±3.6	0±0
NNK	21.3±2.9	0.5±0.8	0.3±0.3	8.5±4.6	7.8±6.4	4.3±3	2.8±3.2	1.3±2	0.8±1.3
Analgin	21.2±4.8	0.8±1.6	0±0	1.7±2.4	3.7±2.6	3.0±1.83.1	1.0±1.7	2.2±3.4	1.3±2.0
Diclofenac	24.8±4.1	0.5±0.8	0±0	1.2±1.8	1.8±1.0	4.5±3.1	1.8±2.4	0.3±0.8	0.3±0.8

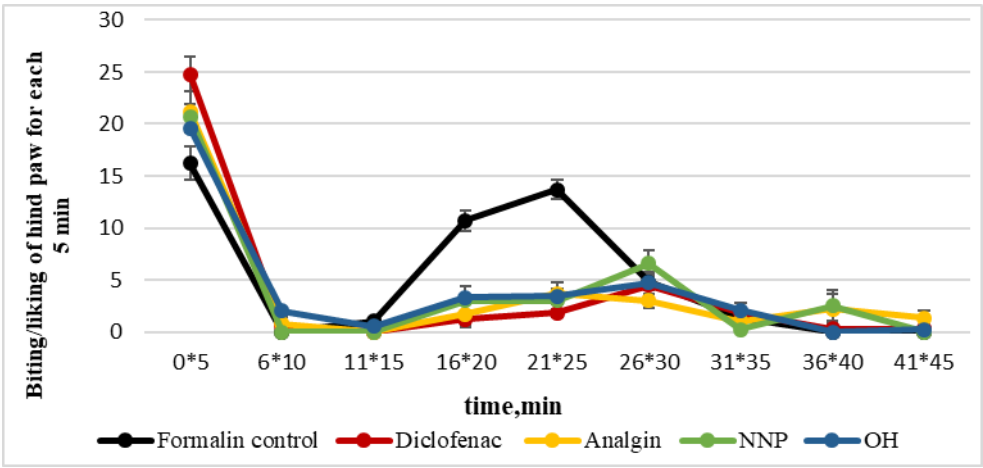

Fig 5. Comparison of the analgesic effect of cobras' venoms with standard analgesics

In the II phase, the NNP venom decreased pain sensitivity by 75.4% (**, $p<0.05$), and the OH venom by 78.3% (**, $p<0.05$), while the analgesic effect of Diclofenac and Analgin in the II phase was 87.7% and 77.9%, respectively.

Discussion

The peculiarity of the present investigation is that the analgesic effects of the venoms of five different cobras were studied under the same conditions, which makes it possible to compare them objectively. It is known from the scientific data that animal keeping conditions, temperature, and diet have a profound effect on experimental data. The fact that cobra venom has an analgesic effect also is well known. However, all of these studies have been done by different authors, under different conditions, on various models of pain, so that data are difficult to compare. The advantage of the present study is that all venoms were tested under the same conditions, on the same model of pain, so it is possible to compare the results to identify the most effective venom for further experimental work.

The total effectiveness of the tested venoms antinociceptive properties during 45min increases in the following sequence: *NNK > NNN > NNO > OH > NNP*, and the effectiveness of the tested venoms antinociceptive properties during inflammatory phase (16*25min) increases in the following sequence: *NNK (33.2%) > NNN (38.5%) > NNO (48.4%) > NNP (75.4%) > OH (78.3%).*

The study results showed that all tested cobras' venoms had an analgesic effect in the second inflammatory phase of the formalin test. NNP venom was 75.4%, and

OH venom: 78.3% effective against the pain. The analgesic capacity of Analgin and Diclofenac under the same conditions were 77.9% and 88.7%, respectively, compared to the control group. Thus, Naja n. pallida and OH venoms or its certain components may be chosen as the most effective from tested venoms for further development of pain relief remedies.

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