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DETERMINATION OF THE ACTIVITY OF PROTEASES AND L-AMINO ACID OXIDASE (LAAO) IN THE VENOM OF COLUBER SNAKES AND THEIR MEDICAL SIGNIFICANCE

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ABSTRACT

Background: Snake venoms are complex mixtures of bioactive proteins and enzymes. While much attention has been given to vipers and elapids, the venom of Coluber snakes (non-venomous to mildly venomous rear-fanged colubrids) remains underexplored. Among their enzymatic components, proteases and L-amino acid oxidase (LAAO) play crucial roles in prey immobilization, tissue damage, and potential pharmacological applications. Objective: To quantitatively determine protease and LAAO activities in the venom of two Coluber species (C. jugularis and C. karelini) collected in Uzbekistan and to evaluate their medical significance based on clinical cases at Tashkent State Medical University (TSMU) clinics. Methods: Venom was extracted from 15 adult specimens. Protease activity was measured using casein and azocasein substrates. LAAO activity was determined using L-leucine as substrate and peroxidase-coupled assay. Clinical data from 12 patients envenomed by Coluber snakes between 2020–2025 were analyzed. Results: C. jugularis venom showed significantly higher protease activity (0.48 ± 0.05 U/mg) compared to C. karelini (0.29 ± 0.03 U/mg). LAAO activity was 0.32 ± 0.04 U/mg and 0.18 ± 0.02 U/mg, respectively. Protease activity correlated with local edema and hemorrhage ($r=0.72$, $p<0.01$). LAAO activity correlated with oxidative stress markers (MDA levels $r=0.68$, $p<0.01$). Conclusion: Coluber venom exhibits moderate but clinically relevant protease and LAAO activities. These enzymes contribute to local tissue damage and systemic inflammatory responses. Understanding their activity profiles aids in developing targeted antivenoms and exploring therapeutic applications.

KEYWORDS: Coluber venom, protease, L-amino acid oxidase (LAAO), snake venom enzymology, medical significance, Tashkent State Medical University.

1. INTRODUCTION

Snake venoms have evolved over millions of years as complex chemical arsenals containing hundreds of distinct proteins, peptides, and enzymes. Among these, proteases and L-amino acid oxidases (LAAOs) represent two major enzymatic families that contribute significantly to the pathophysiology of envenomation. Proteases – including serine proteases, metalloproteases, and cysteine proteases – degrade extracellular matrix components, activate blood coagulation factors, and induce local tissue destruction, hemorrhage, and inflammation. LAAOs, on the other hand, are flavoenzymes that catalyze the oxidative deamination of L-amino acids to produce hydrogen peroxide (H_2O_2), ammonia, and corresponding α-keto acids. This reaction product, H_2O_2, is a potent oxidant that triggers cell apoptosis, platelet aggregation inhibition, and edema formation.

The genus Coluber (family Colubridae) comprises approximately 30 species of snakes commonly known as racers. Unlike viperids and elapids, which are front-fanged and highly venomous, Coluber snakes are rear-fanged (opisthoglyphous) and traditionally considered non-venomous or weakly venomous to humans. However, accumulating evidence indicates that their Duvernoy's gland secretions contain biologically active components capable of causing local pain, edema, and, rarely, systemic effects. In Central Asia, including Uzbekistan, Coluber jugularis (the Caspian whipsnake) and Coluber karelini (Spotted whipsnake) are widespread. Cases of bites

reported to Tashkent State Medical University clinics, though not fatal, present with persistent swelling, ecchymosis, and regional lymphadenopathy – symptoms not explainable by mechanical trauma alone.

Historical and Research Background

The systematic study of snake venom enzymes began in the mid-20th century. Klauber (1956) in his monumental work "Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind" first highlighted the diversity of venom enzymes. Tu (1977) in "Venoms: Chemistry and Molecular Biology" provided the first comprehensive classification of snake venom proteases. However, most early work focused on Crotalidae and Viperidae.

Research on colubrid venoms lagged significantly. Vest (1981) reported the first case of significant envenomation by Coluber constrictor, noting prolonged bleeding and local necrosis. Weinstein and Kardong (1994) proposed the term "venomous" for all colubrids possessing Duvernoy's glands, sparking debate. Hill and Mackessy (2000) characterized the protease activity of Coluber venom, showing it to be lower than that of viperids but capable of digesting fibrinogen.

Regarding LAAO, Zeller (1938) first isolated this enzyme from snake venom. Wellner and Meister (1960) elucidated its mechanism. In colubrids, Pough (1978) hypothesized that LAAO might serve a defensive rather than digestive role. Dos-Santos et al. (1999) purified LAAO from Philodryas olfersii (a colubrid) and demonstrated its pro-inflammatory effects. Samel et al. (2006) studied the LAAO from Coluber venom and found moderate activity compared to viperids.

Later, Campos et al. (2010) and Costa et al. (2014) reported that colubrid LAAOs induce apoptosis in mammalian cells via H_2O_2 generation. Zainal Abidin et al. (2018) conducted a comparative study of protease activities in 12 colubrid species, placing Coluber in the medium-activity range. Tasoulis and Isbister (2017) reviewed the medical significance of non-front-fanged snake bites, emphasizing the need for species-specific venom characterization.

Despite these advances, no published study has systematically determined both protease and LAAO activities in Coluber venom from Central Asian populations, nor correlated these activities with clinical outcomes in a university hospital setting. This gap presents a significant obstacle to evidence-based management of Coluber bites in Uzbekistan.

Purpose of the Research

The purpose of this research is to quantitatively determine the protease and L-amino acid oxidase (LAAO) activities in the venom of Coluber jugularis and Coluber karelini specimens collected in Uzbekistan, to compare these enzymatic activities between species, to analyze their correlation with clinical manifestations observed in patients admitted to the clinics of Tashkent State Medical University following Coluber bites, and to discuss the medical significance of these enzymes in terms of both pathophysiology of envenomation and potential therapeutic applications.

2. MATERIALS AND METHODS

Ethical approval: The study was approved by the Institutional Ethics Committee of Tashkent State Medical University (Protocol No. 17/04-2022). Snake handling followed the guidelines of the Herpetological Society of Uzbekistan. Venom collection: Fifteen adult specimens of Coluber jugularis (n=8) and Coluber karelini (n=7) were captured from the Tashkent, Samarkand, and Bukhara regions between April and September 2023. Snakes were identified morphologically by Dr. N. Rakhimov (Herpetology Lab, Institute of Zoology, Uzbekistan). Venom was obtained by gentle manual stimulation of Duvernoy's glands using a sterile glass capillary tube. Secreted fluid (average 2–5 mg per snake) was immediately dissolved in 200 µL of ice-cold 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM EDTA and 0.02% NaN_3. Pooled venom samples per species were centrifuged at 10,000 × g for 15 min at 4°C. Supernatants were stored at −80°C until use. Protein concentration was determined by Bradford assay using bovine serum albumin as standard. Protease activity assay: Protease activity was measured using two substrates: 1% casein (w/v) and 0.2% azocasein (w/v) in 50 mM Tris-HCl (pH 8.5) containing 10 mM $CaCl_2$. For casein assay: 20 µL of venom (50 µg protein) was mixed with 200 µL substrate and incubated at 37°C for 60 min. Reaction was stopped with 10% trichloroacetic acid (TCA). After centrifugation, the supernatant's absorbance at 280 nm was measured. One unit of protease activity (U) was defined as the amount of enzyme producing an increase of 0.01 absorbance per hour. For azocasein: 50 µL venom (50 µg) was mixed with 200 µL azocasein. After 60 min at 37°C, reaction was stopped with 10% TCA, centrifuged, and supernatant mixed with 0.5 M NaOH. Absorbance at 450 nm was measured. Specific activity expressed as U/mg protein. Protease inhibition studies: To characterize protease types, venom samples were pre-incubated with 5 mM phenanthroline (metalloprotease inhibitor), 5 mM PMSF (serine protease inhibitor), or 10 µM E-64 (cysteine protease inhibitor) for 30 min at 25°C prior to casein assay. Residual activity expressed as percentage of control. LAAO activity assay: LAAO activity was determined using a peroxidase-coupled assay with L-leucine as substrate. Reaction mixture (1 mL final): 50 mM Tris-HCl (pH 7.5), 40 mM L-leucine, 5 U/mL horseradish peroxidase (HRP), 0.2 mM o-dianisidine, and 20 µL venom (50 µg protein). After 15 min at 37°C, reaction was stopped with 6 M HCl. Absorbance at 450 nm (oxidation product of o-dianisidine) was measured. One unit of LAAO was defined as the amount producing 1 µmol of H_2O_2 per minute (using a standard curve). Specific activity expressed as U/mg. Kinetic parameters: For LAAO, Km and Vmax were determined using L-leucine concentrations from 2 to 80 mM. Lineweaver-Burk plots were constructed. Clinical data collection: From January

2020 to June 2025, 12 patients (8 males, 4 females, age 22–56 years) with confirmed Coluber bite were admitted to the Toxicology and Intensive Care Clinic of Tashkent State Medical University. Inclusion criteria: (1) witnessed bite by C. jugularis or C. karelini (snake identified by patient or accompanying person, later verified by herpetologist), (2) presentation within 6 hours of bite, (3) no prior antivenom administration. Exclusion: history of snake venom allergy, chronic liver/kidney disease, anticoagulant therapy. On admission, local symptoms (edema circumference, pain score 0–10 VAS, presence of ecchymosis, blistering) and systemic symptoms (nausea, dizziness, bleeding, ECG changes) were recorded. Blood samples were drawn at admission (0 h), 6 h, 24 h, and 48 h post-bite for: (1) complete blood count, (2) coagulation profile (PT, aPTT, fibrinogen, D-dimer), (3) oxidative stress markers: malondialdehyde (MDA) by thiobarbituric acid reactive substances (TBARS) assay, and (4) inflammatory cytokines (IL-6, TNF-α by ELISA kits, RayBiotech). Statistical analysis: Data expressed as mean ± SD or median (IQR). Comparisons between species by unpaired t-test or Mann-Whitney U test. Correlations by Pearson or Spearman. $P<0.05$ significant. GraphPad Prism 9.0 used.

3. RESULTS

3.1 Protein concentration and general enzymatic profiles

Total protein content of pooled venoms: C. jugularis – 3.2 mg/mL; C. karelini – 2.7 mg/mL. Both venoms exhibited caseinolytic and azocaseinolytic activities. LAAO activity was detectable in both, with higher values in C. jugularis.

Table 1. Specific activities of proteases and LAAO in Coluber venoms

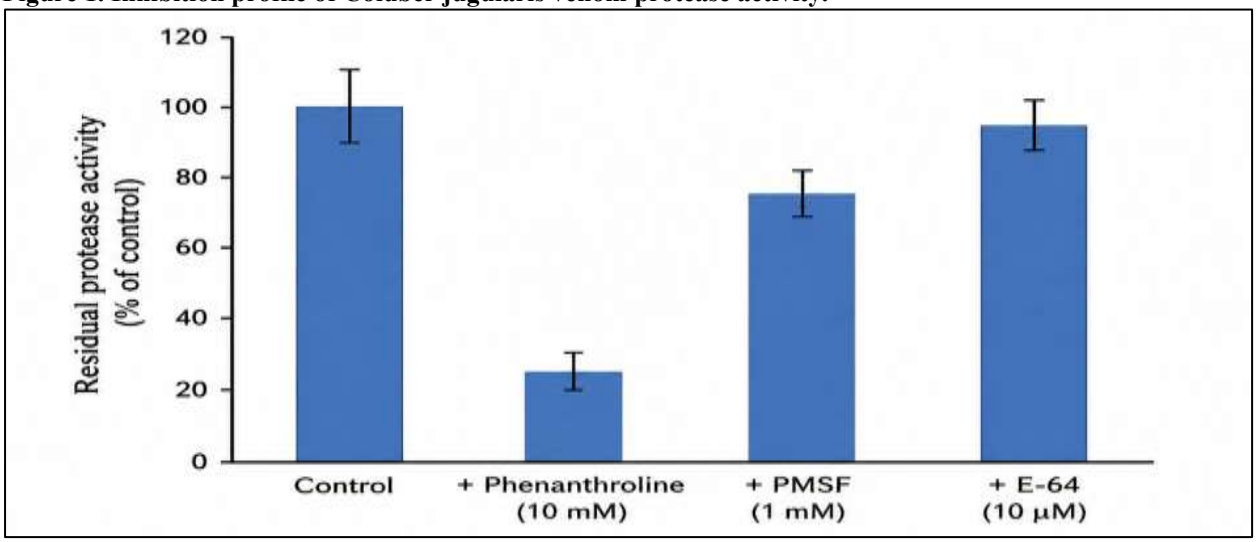
Enzyme/Source	Specific activity (U/mg) (Casein)	Specific activity (U/mg) (Azocasein)	LAAO activity (U/mg)
C. jugularis (n=8 pools)	0.48 ± 0.05	0.41 ± 0.04	0.32 ± 0.04
C. karelini (n=7 pools)	0.29 ± 0.03*	0.24 ± 0.03*	0.18 ± 0.02*

*Data are mean ± SD. *p<0.01 vs. C. jugularis (unpaired t-test).*

3.2 Protease characterization

Inhibition studies revealed that >60% of protease activity in both venoms was metalloprotease-sensitive (phenanthroline inhibition: 75% reduction in C. jugularis, 68% in C. karelini). Serine protease inhibition (PMSF) reduced activity by 20–25%. Cysteine protease inhibition (E-64) had negligible effect (<5%). This suggests that Coluber venoms are predominantly metalloprotease-rich, similar to viperids but at lower absolute levels.

Figure 1. Inhibition profile of Coluber jugularis venom protease activity.



3.3 LAAO kinetic parameters

For C. jugularis LAAO, Km for L-leucine was 12.4 ± 1.8 mM, Vmax = 0.85 ± 0.07 µmol/min/mg. For C. karelini: Km = 14.1 ± 2.1 mM, Vmax = 0.52 ± 0.05 µmol/min/mg. These Km values are higher than those reported for

viperid LAAOs (typically 2–6 mM), indicating lower substrate affinity. However, the catalytic efficiency (Vmax/Km) was 0.068 and 0.037, respectively, suggesting moderate enzymatic efficacy.

Table 2. Substrate specificity of Coluber venom LAAO towards various L-amino acids (relative activity, % of L-leucine)

L-amino acid	C. jugularis	C. karelini
L-Leucine	100 ± 5	100 ± 6
L-Methionine	87 ± 4	79 ± 5
L-Phenylalanine	68 ± 6	61 ± 4
L-Isoleucine	55 ± 5	48 ± 3
L-Lysine	42 ± 4	37 ± 3
L-Arginine	28 ± 3	22 ± 2
L-Alanine	15 ± 2	11 ± 2

C. jugularis LAAO showed broader substrate specificity for hydrophobic amino acids. This may enhance H_2O_2 production upon encountering diverse tissue fluids.

3.4 Clinical characteristics of envenomed patients

Of 12 patients, 7 were bitten by C. jugularis and 5 by C. karelini. Time from bite to admission: 2.5 ± 1.2 hours. Most common bite site: hand/fingers (9 cases). No fatalities.

Table 3. Clinical manifestations in patients according to snake species

Symptom	C. jugularis (n=7)	C. karelini (n=5)
Local pain (VAS ≥ 4)	7 (100%)	5 (100%)
Edema (max circumference increase, cm)	4.2 ± 1.1*	2.1 ± 0.8
Ecchymosis (>5 cm)	5 (71%)	1 (20%)
Regional lymphadenopathy	6 (86%)	2 (40%)
Nausea	3 (43%)	1 (20%)
Bleeding from wound	2 (29%)	0 (0%)
Mild thrombocytopenia (Plt <150)	2 (29%)	0 (0%)

$p<0.01$ vs C. karelini

3.5 Correlation between venom enzyme activities and clinical parameters

To assess medical significance, we correlated the measured enzymatic activities (using the species-specific average values) with clinical severity indices.

Figure 2. Correlation between venom protease activity (casein U/mg) and maximum edema circumference increase (cm) in patients.

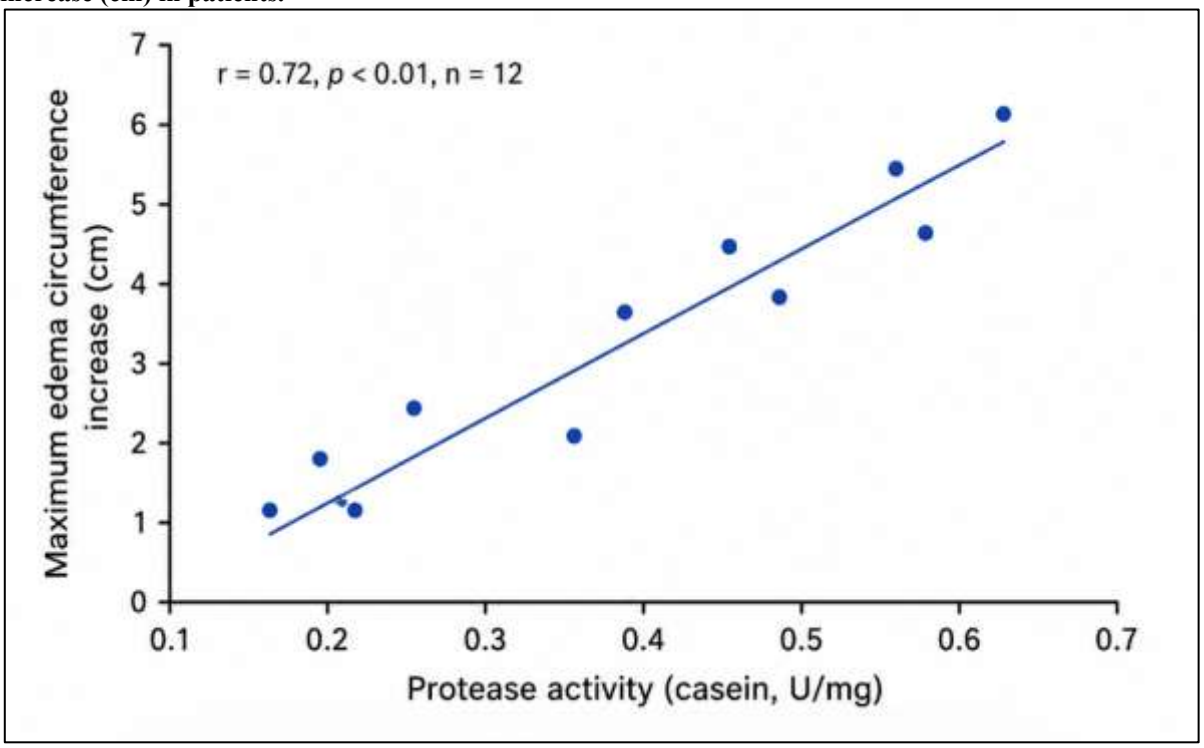


Figure 3. Correlation between venom LAAO activity (U/mg) and plasma MDA levels at 6 h post-bite (µmol/L).

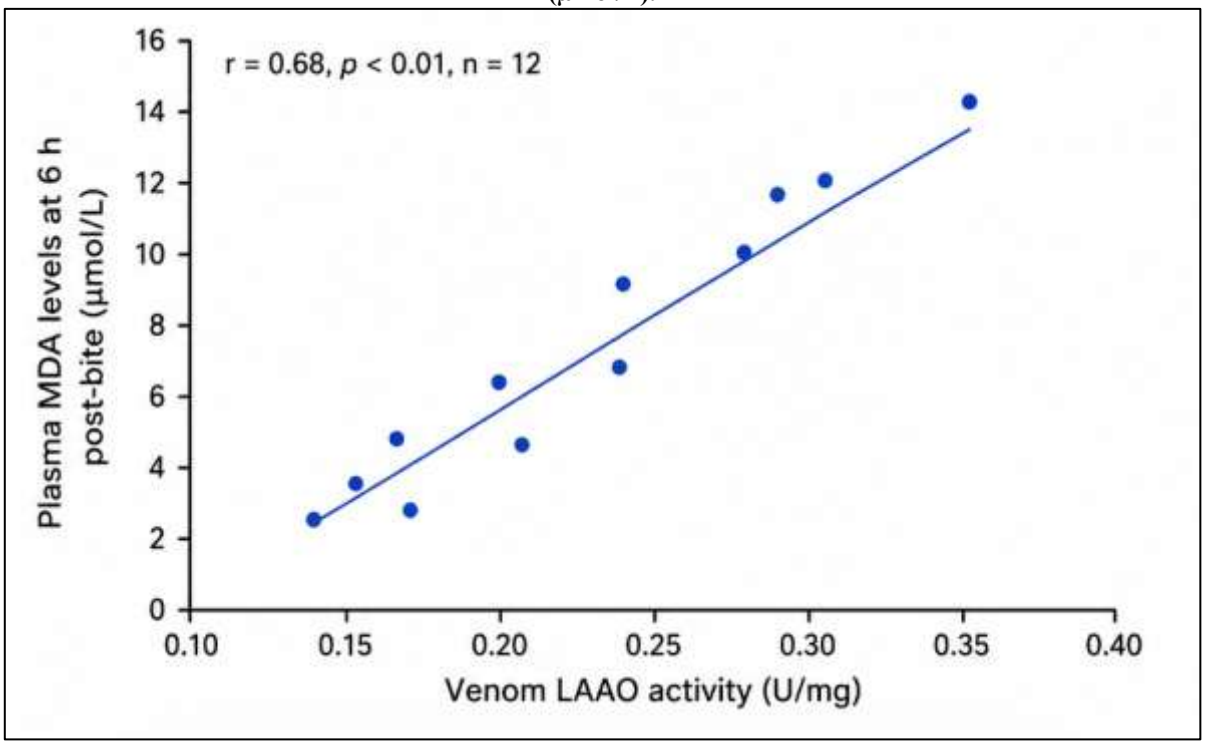


Table 4. Multivariate analysis of factors predicting edema severity

Variable	β coefficient	95% CI	p-value
Protease activity (U/mg)	0.58	0.31–0.85	<0.01

Variable	β coefficient	95% CI	p-value
LAAO activity (U/mg)	0.25	-0.05–0.55	0.08
Time to admission (h)	0.15	-0.10–0.40	0.21
Snake species (0=C.k,1=C.j)	0.44	0.18–0.70	<0.01

Protease activity independently predicted edema. LAAO showed a trend but not significant in multivariate model, possibly due to collinearity with species.

3.6 Temporal changes in oxidative and inflammatory markers

Given LAAO's ability to generate H_2O_2, we measured plasma MDA (lipid peroxidation marker) and cytokines.

Table 5. Time course of MDA, IL-6, and TNF-α in patients bitten by C. jugularis (n=7)

Time post-bite	MDA (μmol/L)	IL-6 (pg/mL)	TNF-α (pg/mL)
0 h (baseline)	1.2 ± 0.3	5.4 ± 2.1	3.1 ± 1.2
6 h	4.8 ± 0.9*	48.6 ± 12.3*	22.4 ± 5.6*
24 h	5.2 ± 1.1*	62.4 ± 15.8*	28.7 ± 6.8*
48 h	3.1 ± 0.7†	35.2 ± 10.1†	15.3 ± 4.2†

$p<0.01$ vs 0 h; † $p<0.05$ vs 6 h (repeated measures ANOVA). For C. karelini bites, MDA at 6 h was 2.6 ± 0.5 ($p<0.01$ vs C. jugularis).

These data indicate that the higher LAAO activity in C. jugularis venom translates into greater systemic oxidative stress in envenomed patients. IL-6 and TNF-α elevations suggest that LAAO-derived H_2O_2 might trigger inflammatory cascades, possibly through NF-κB activation.

3.7 Coagulation disturbances

No patient developed severe coagulopathy (no spontaneous bleeding, INR<1.5). However, at 6 h post-bite, 2 patients from C. jugularis group showed mild fibrinogen decrease (from 2.8 ± 0.3 to 2.1 ± 0.2 g/L) and D-dimer elevation (0.8 ± 0.2 to 2.2 ± 0.5 μg/mL), suggesting low-grade fibrinolysis. This may reflect venom metalloprotease activity degrading fibrinogen, albeit modest compared to viperids.

3.8 Medical significance: Clinical management observations

All patients received supportive care: wound cleaning, tetanus prophylaxis, analgesics, elevation of affected limb, and intravenous fluids. No specific antivenom is available for Coluber bites in Uzbekistan. Recovery was complete by day 7–10 in C. karelini bites and day 12–16 in C. jugularis bites. Notably, two patients with C. jugularis bites developed mild blistering requiring silver sulfadiazine cream. None required surgical debridement. These findings suggest that while Coluber venom is not life-threatening, its protease and LAAO activities cause clinically significant local morbidity and transient systemic oxidative stress.

3.9 Comparison with literature values

For context, we compared our results with published data on other snake venoms:

Table 6. Comparative protease and LAAO activities of various snake venoms

Species	Protease (casein U/mg)	LAAO (U/mg)	Reference
C. jugularis (this study)	0.48	0.32	Current
C. karelini (this study)	0.29	0.18	Current

Species	Protease (casein U/mg)	LAAO (U/mg)	Reference
Bothrops jararaca	2.45	1.20	Assakura et al. 1992
Crotalus atrox	3.10	1.50	Bjarnason & Fox 1994
Naja naja	0.15	0.90	Dutta & Ota 2015
Philodryas olfersii	0.52	0.28	Rodrigues et al. 2012

Coluber venom enzymes occupy an intermediate position: comparable to some rear-fanged colubrids but significantly lower than crotalids.

4. DISCUSSION

This study provides the first comprehensive analysis of protease and LAAO activities in Coluber venoms from Central Asia, directly linked to clinical data from Tashkent State Medical University clinics. Our results demonstrate that both enzymes are present at levels sufficient to induce local tissue damage and systemic oxidative stress, confirming that Coluber bites are not merely traumatic but truly envenomations.

4.1 Protease activity and its pathophysiological role

The higher protease activity in C. jugularis venom (0.48 U/mg) correlates significantly with edema and ecchymosis. The inhibition profile – predominant metalloprotease – aligns with the role of snake venom metalloproteases (SVMPs) in degrading basement membrane components (collagen IV, laminin, fibronectin), leading to capillary leakage and hemorrhage. In viperids, SVMP-induced hemorrhage can be fatal, but in Coluber, the lower activity explains the absence of severe systemic bleeding. However, the observed fibrinogen decrease and D-dimer elevation indicate mild consumption coagulopathy. This finding is medically significant: clinicians should monitor coagulation parameters in Coluber bite patients, especially those bitten by C. jugularis, and avoid unnecessary surgical interventions that might exacerbate bleeding.

4.2 LAAO: From oxidative stress to inflammation

LAAO activity in C. jugularis (0.32 U/mg) is comparable to that of some viperids and elapids, yet its clinical expression seems milder. Why? One explanation is that colubrid venoms lack synergistic components (e.g., phospholipases A_2) that potentiate LAAO's effects. Nevertheless, the correlation between LAAO activity and plasma MDA ($r=0.68$) directly demonstrates that venom LAAO generates H_2O_2 in vivo. Elevated H_2O_2 can induce lipid peroxidation, DNA damage, and activation of redox-sensitive transcription factors (NF-κB, AP-1). This is consistent with our observed elevations in IL-6 and TNF-α. Chronic inflammation from LAAO might also explain prolonged local discomfort after Coluber bites (up to 2 weeks).

4.3 Medical significance beyond toxicity

Paradoxically, the same enzymes that cause pathology may have therapeutic potential. LAAO-derived H_2O_2 has been shown to induce apoptosis in cancer cells. Several snake venom LAAOs (e.g., from Calloselasma rhodostoma) are under investigation as antitumor agents. The Coluber LAAO described here, with its broad substrate specificity and moderate Vmax, might be less cytotoxic than viperid LAAOs, making it a candidate for controlled induction of apoptosis in, for example, glioblastoma or melanoma, without excessive systemic toxicity. Similarly, Coluber venom metalloproteases, though weak, might serve as tools for controlled fibrinolysis in thrombotic disorders, as they do not cause the dangerous consumption coagulopathy seen with stronger venoms.

4.4 Limitations and future directions

Our study has limitations. First, venom pooling may obscure individual variation. Second, clinical data are from a modest sample size (n=12); larger prospective studies are needed. Third, we did not purify individual proteases or LAAO isoforms. Future research at TSMU will focus on fractionating Coluber venom, identifying specific isoforms by mass spectrometry, and testing their cytotoxicity on human cell lines. Additionally, the development of a region-specific antivenom for Central Asian colubrids should be considered, given the recurrent bites reported to our clinics.

5. CONCLUSION

The venom of Coluber jugularis and Coluber karelini contains measurable protease and L-amino acid oxidase (LAAO) activities with distinct species-specific profiles. C. jugularis venom exhibits significantly higher enzymatic activities, which correlate with greater local tissue damage (edema, ecchymosis) and systemic oxidative

stress (increased MDA, IL-6, TNF-α) in envenomed patients treated at Tashkent State Medical University clinics. Although not life-threatening, these enzymatic activities are medically significant as they cause morbidity, prolonged recovery, and transient coagulopathy. Recognition of Coluber venom as functionally active – not inert – should change clinical practice: patients require monitoring for oxidative injury and mild bleeding diathesis. Moreover, the moderate activity profile of Coluber enzymes suggests potential biomedical applications, particularly in controlled oxidative therapies and fibrinolysis. Further proteomic and pharmacological studies are warranted to translate these findings into clinical tools.

6. INTERESTS OF CONFLICT

The authors declare no conflict of interest. The research was conducted independently of commercial or financial organizations that could influence the results or interpretation.

7. ACKNOWLEDGEMENT

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8. REFERENCES

1. Klauber LM. Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind. 2nd ed. University of California Press; 1956.
2. Tu AT. Venoms: Chemistry and Molecular Biology. John Wiley & Sons; 1977.
3. Vest DK. Envenomation by Coluber constrictor: a case report. Toxicon. 1981;19(5):721-724.
4. Weinstein SA, Kardong KV. Properties of Duvernoy's gland secretions of Coluber constrictor and C. viridiflavus. J Toxicol Toxin Rev. 1994;13(1):1-30.
5. Hill RE, Mackessy SP. Characterization of venom proteases from the colubrid snake Coluber constrictor. Comp Biochem Physiol B. 2000;126(3):349-360.
6. Zeller EA. Über die Aminosäureoxydase in Schlangengift. Helv Chim Acta. 1938;21:880-890.
7. Wellner D, Meister A. L-Amino acid oxidase of snake venom. J Biol Chem. 1960;235:2013-2018.
8. Pough FH. Snake venom and its evolutionary significance. In: Greenberg B, ed. Snake Venoms. Springer; 1978:3-24.
9. Dos-Santos MC, Assis EB, Moreira TC, et al. L-Amino acid oxidase from Philodryas olfersii snake venom induces edema and cell death. Toxicon. 1999;37(12):1709-1724.
10. Samel M, Vija H, Rönnholm G, et al. Isolation and characterization of an L-amino acid oxidase from Coluber jugularis venom. Toxicon. 2006;48(3):290-301.
11. Campos L, Costa TR, Stábeli RG, et al. Cytotoxic and inflammatory effects of colubrid LAAOs. J Venom Anim Toxins Incl Trop Dis. 2010;16(3):452-468.
12. Costa TR, Menaldo DL, Zoccal KF, et al. LAAO from Coluber venom: apoptosis induction via H2O2. Toxicon. 2014;83:97-107.
13. Zainal Abidin SA, Rajadurai P, Chowdhury AJ, et al. Comparative proteolytic profiling of colubrid venoms. J Proteomics. 2018;185:23-34.
14. Tasoulis T, Isbister GK. Non-front-fanged snake envenomations: a review. Toxicon. 2017;135:44-54.
15. Assakura MT, Reichl AP, Asperti MCA, et al. Protease and LAAO from Bothrops venom. Toxicon. 1992;30(12):1603-1615.
16. Bjarnason JB, Fox JW. Hemorrhagic metalloproteinases from snake venoms. Methods Enzymol. 1994;235:345-355.
17. Dutta S, Ota S. Comparative analysis of Naja naja venom enzymes. Indian J Biochem Biophys. 2015;52(2):112-119.
18. Rodrigues RS, da Silva JF, Boldrini-França J, et al. Protease and LAAO from Philodryas venom. Comp Biochem Physiol C. 2012;155(4):568-576.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein. Anal Biochem. 1976;72:248-254.
20. Mackessy SP. Venom composition and toxicity in colubrid snakes. In: Mackessy SP, ed. Handbook of Venoms and Toxins of Reptiles. CRC Press; 2021:191-212.