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SNAKE VENOM'S BITE AGAINST ORAL CANCER: A THERAPEUTIC FRONTIER

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Abstract

Oral squamous cell carcinoma (OSCC) stands as a prevalent malignancy, accounting for a significant burden worldwide, particularly in developing nations. Current treatment modalities, while diverse, often exhibit non-specific cell death and present adverse effects, underscoring the need for alternative, more targeted therapies. Amid this quest, natural bioactive substances have emerged as promising candidates, with snake venom attracting attention for their diverse compounds and potential therapeutic effects. This review explores the intricate landscape of snake venom and their applications in OSCC treatment. Snake venoms, intricate mixtures comprising proteins, peptides, and bioactive elements, exhibit varied toxic actions and composition across different snake families. While early studies on crude snake venoms showed limited promises in tumor inhibition, subsequent investigations pinpointed specific compounds with targeted effects. Studies focusing on venom-derived compounds have elucidated their potential mechanisms of action against OSCC. Compounds like Cardiotoxin III (CTXIII) and Crotoxin have shown promising anti-proliferative, pro-apoptotic, and anti-migratory effects in OSCC cell lines. Mechanistic insights reveal their ability to modulate signaling pathways associated with angiogenesis inhibition, apoptosis induction, and cell cycle arrest, offering potential avenues for targeted OSCC therapy. Additionally, commercially available snake toxin-based products showcase the therapeutic potential of these compounds across various medical contexts, presenting a promising frontier for further exploration in OSCC treatment. This comprehensive review underscores the potential of snake venom-derived compounds as adjunct therapies for OSCC, emphasizing the need for continued research and development in harnessing these natural bioactive substances. The journey from crude venom applications to compounds targeting specific pathways marks a promising trajectory, presenting a compelling avenue for the future of OSCC treatment strategies.

Keywords: Snake Venom, OSCC, Cardiotoxin III, Malignancy, Bioactive Elements.

INTRODUCTION

Oral cancer is a malignant condition that primarily affects the lip or oral cavity. 90% of oral cancer is called OSCC (oral squamous cell carcinoma) as the histological origin is squamous cells (1). It is the most prevalent malignancies in the world with a high specificity in developing countries (2). Clinically the first changes seen are oral potentially malignant disorders commonly leukoplakia and erythroplakia. Microscopically the changes seen are non-aberrant keratinocytes followed by

hyperplasia of epithelial cells, different degree of dysplasia, leading to a carcinoma in situ and finally converted into an invasive carcinoma (3). The most common potential risk factors include tobacco, smoking, and alcohol. Currently there are many treatment modalities for effective treatment such as chemoradiotherapy, surgery, EGFR inhibitors and COX-2 inhibitors, and photodynamic therapy. However, the major drawback seen with all modalities is non-specific cell death (4). It has been seen that a few natural products targeting different pathways and signaling molecules may promote oral cancer treatment (5). In this article, snake venom used for OSCC therapy has been discussed. Conventional treatment modalities for oral cancer led to several adverse effects sometimes fatality (4). Thus, in recent times there has been a turn towards natural bioactive substitutes as an alternative for the conventional modalities to as to reduce the adverse effects thereby giving rise to a safe option for treatment (5). One group of compounds being intensively studied and reported in literature is snake venoms. These derivates show a promise as an adjunct to traditional modalities of treatment by reducing the harsh consequences and adverse effects. Therefore, this present review highlights and discusses snake venoms and their mechanisms of action in the treatment of OSCC.

Snake venoms is secreted by the venom gland of snakes and are complex mixtures consisting of proteins, peptides, and bioactive substances (6). They cause a variety of toxic actions such as neurotoxicity, cyto- toxicity, cardiotoxicity, myotoxicity along with certain enzymatic activities (7). Venomous snakes are seen in many families including colubris, elapid, viperid. The composition of venoms varies within the different families (8). The composition of venoms of the common families has been summarized in Table 1. (9)

Table 1: Composition of venoms from colubrid, elapid and viperid snakes

Composition	Colubrid (e.g. Boiga dendrophilia)	Elapid (e.g. D. angusticeps, N. nivea, cobra, mamba)	Viperid (e.g. B.jararaca, B. atrox, rattlesnake)
Non-enzymatic proteins/ peptides			
3 finger toxins, α-neurotoxin	+	+	+
CRISPs	+	+	+
Bradykinin-potentiating factor	–	–	+
Disintegrins	–	–	+
Myotoxins	–	–	+
Enzymes			
Proteases	+	+	+
Phospholipase A2	+	+	+
Pre-synaptic phospholipase A2 neurotoxins	–	+	+

METHODOLOGY

The methodology for this comprehensive review on "Snake Venom's Bite Against Oral Cancer: A Therapeutic Frontier" involves a systematic approach utilizing Pubmed and Scopus databases. The search strategy will employ Boolean operators, incorporating keywords and MeSH terms related to snake venom, oral cancer, therapeutic effects, and treatment. Inclusion criteria encompass peer-reviewed English publications, focusing on the therapeutic potential of snake venom against oral cancer, including

research articles, clinical trials, reviews, and meta-analyses. Following an initial search and subsequent filtering based on publication date and relevance, screening will occur based on titles and abstracts, leading to the retrieval of potentially relevant full-text articles. Selection of 25 articles meeting the criteria will involve a meticulous assessment of the full texts, extracting pertinent data such as author information, study design, venom species involved, mechanisms of action, clinical outcomes, and limitations. The synthesized information will be analysed to highlight patterns, trends, and the potential application of snake venom in oral cancer treatment, ultimately resulting in a coherent narrative presenting the current state of research in this domain.

In 1930s, the effect of crude snake venom was first studied and while positive clinical effects were seen in the laboratory in studies by Essex et al. (10) and Kurotchkin et al. (11), no promising effects were seen in humans. Later studies showed no tumor suppression results in humans but analgesic effects as seen in studies by Macht et al. (12) On the hind-side a hotspot developed for the enzymatic studies of snake venoms. To summarize, crude snake venom proved not very hopeful in the inhibition of tumor cells, but pain relief was their main effect when used as a mixture in patients with hopeless prognosis of tumors.

Later studies such as that by Liu et al. showed that poisonous substances have a targeted anti-tumor effect on tumor cells and thus may use as treatment modalities for cancer treatment (13).

Antitumor activities of Snake Venom

Growth of human tumor requires neovascularization for the essential oxygen and nutrient supply. Anti-tumor activities thus are broadly classified into antiangiogenesis and apoptosis induction which are facilitated by various snake venom compounds summarized in table 2 (14).

Table 2: Compounds with antitumor activites isolated from snake venoms.

Mechanism	Protein - Compound	Snake species
Antiangiogenesis	Leucurogin - disintegrin	Bothrops leucurus
	Contortrostatin - disintegrin	Agkistrodon contortrix contortrix
	Obtustatin - disintegrin	Vipera lenetina obtusa
	Adinbitor - disintegrin	A.halys brevicadus stejneger
	Salmosin - disintegrin	A.halys brevicadus
Apoptosis induction	LAAO – LAAO	A.halys
	AHP-LAAO – LAAO	A.halys pallas
	LAAO - LAAO	V.berus berus
	Disintegrin - disintegrin	Naja naja
	VAP and VAP2 – MMP/ disintegrin	Crotalus atrox
	Stejnitin - SVMP	Trimeresurus stejnegeri

Isolation of snake venom from Naja naja atra (Formosan cobra) gives Cardiotoxin III (CTXIII).(15) CTXIII was found to play a role in the inhibition of proliferation of cells and apoptotic induction in various cancer cells such as breast cancer (16), leukemia (17), colorectal cancer(18), and oral cancer (19,20)

A study by Ching- Ming Chein et al. showed that CTXIII shoes significant apoptotic induction in Ca9-22 cells by the endothelial growth factor mediated pathway. Treatment of CTXIII in Ca9-22 cells reduced the phosphorylation of endothelial growth factor receptor, caused an inhibition of STAT 3 and STAT 5 activation in time dependant manner. A down regulation of anti-apoptotic proteins such as Bcl2, Bcl-X

and myeloid leukemia – 1 with an up regulation of Bax was seen. Due to treatment, there was a mitochondrial membrane disruption thereby causing a release in of mitochondrial cytochrome c followed by caspases 9 and 3. Thus this study showed that CTXIII has potential as a therapeutic effect against Ca9-22 OSCC cells (19).

A study carried out by Ching- Ming Chein et al. resulted in indication that CTXIII inhibits Src kinase causing apoptosis and S-phase arrest. CTXIII showed toxicity leading to S-phase cell cycle arrest which was seen via cyclin A, cyclin B, and cyclin-dependent kinase 1 (CDK1), and apoptosis expression decrease. Along with that there has been shown Bcl-2, p-Bad, and X-linked inhibitor of apoptosis (XIAP) downregulation and an upregulation of Bax and Bad. There is release of cytochrome C along with caspase-9 and caspase-3 in Ca9-22 cells activation sequentially. All together it results as arrest of S-phase along with apoptosis in Ca9-22 cells due to an inactivation of Src, EGFR, STAT3, STAT5, PI3 K(p110), and Akt signalling pathways (20).

A study by Yen et al. showed that there is a role of p38-MAPK and MMP-2/-9 pathways in the inhibition effect of proliferation and migration by CTXIII treatment in human oral cancer cells. An antimigration potential was exerted against Ca9-22 cells by CTXIII in a dose-response manner. This leads to activation of p-JNK and p-38 without any effect on ERK signal. This cause MMP 2 and 9 downregulation. Thus, this results in Ca9-22 cell migration potential attenuation. (15)

A study by Rogério Gonçalves da Rocha et al. showed the effects of crotoxin on oral cancer cell lines and animal model chemically infected with oral cancer. Crotoxin is isolated from the venom of South American Crotalus durissus terrificus. This treatment significantly reduced the frequency of OSCC by 50%. (21)

In a study by Lin Chai et al. the mechanism of component I from agkistrodon acutus venom (AAVC-I) was studied for apoptotic effects of OSCC. The component I (AAVC-I) showed apoptosis of HN-4 cells in a concentration dependant manner. This was followed by mitochondrial membrane depolarization and cellular apoptosis. (22)

DISCUSSION

Commercially available snake toxin based therapeutic modalities.

Extensive research has now produced many snakes' toxin-based modalities that are available for use. Most toxin-based drug products are isolate from snakes primarily due to the numerous types and large amounts of venoms produced. (24,25) The commercially available snake toxin-based products are summarized in table 3. There is however still huge lacunae present in for studies of snake toxins specifically as treatment modalities for oral cancer.

Table 3: Commercially available snake toxin based therapeutic molecules (23)

Molecules	Species	Mechanism of action	Clinical Use/ Cell lines used
Batroxobin (de fibrase)	Brazilian lancehead snake	Cleaves Aα-chain of fibrinogen	Microvascular thrombosis
Batroxobin (plateltex-Act)	Common lancehead snake	Gelification of blood	Human mesenchymal stem cells
Batroxobin – Fibrin sealant (Vivostat)	Brazilian lancehead snake	Cleaves Aα-chain of fibrinogen	Autologous fibrin sealant in surgery

Cobratide (Ketonging, cobrotoxin)	Chinese cobra	Blockage of nicotinic receptors	Chronic arthralgia, sciatica, neuropathic headache
Captopril (Capoten)	Jararaca pit viper snake	Angiotensin-converting enzyme inhibitor	Hypertension, cardiac failure
Enalapril (Vasotec)	Jararaca pit viper snake	Angiotensin-converting enzyme inhibitor	Hypertension, cardiac failure
Eptifibatide (Integrillin)	Pigmy rattlesnake	Prevents binding of fibrinogen, von Willebrand factor, other adhesive ligands to GPIIb/IIIa	Acute coronary syndrome, percutanerous coronary intervention
Tirofiban (Aggrastat)	Saw scaled viper snake	Antagonist of fibrinogen binding to GPIIb/IIIa	Acute coronary syndrome

In exploring the therapeutic potential of snake venom against oral cancer, envision a transformative landscape where global scientific collaboration unfolds within the metaverse. Within this virtual laboratory, scientists transcend geographical boundaries, sharing discoveries, simulating scenarios, and ideating on the utilization of specific venom compounds for combating oral cancer. This digital realm fosters real-time, borderless cooperation, expediting the pace of research and discovery. Concurrently, the implementation of blockchain technology emerges as a guardian of data integrity in this scientific pursuit. Blockchain ensures the secure management and verification of extensive venom research data, including compound specifics, clinical trial information, and treatment outcomes. By offering a transparent and tamper-proof system, blockchain cultivates trust among researchers, pharmaceutical entities, and patients by curbing the risks of data manipulation and fraud (26).

CONCLUSION

Compound derived from natural sources show immense potential as therapeutic agents in the treatment modalities of oral cancer. These are now a hotspot for research as traditional treatment modalities for cancer have many serious adverse effects. Other naturally derived compounds such as green tea catechins, panax ginseng, polygonum cuspidatum also prevent cancer. Application of snake venom to cancer have gone from crude venom applications to components that target specific pathways. Combination of snake venoms with other technologies such as nanoparticles is the future in cancer treatment though current research is at a very initial stage. The various studies of snake venoms derivatives for treatment of oral cancer are mentioned in this article. Thus, compounds from natural source especially snake venom derivative studies should be the future in research of oral cancer.

References

1) Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral Oncol.* 2008;44:10–22.

2) Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Dicker D, Pain A, et al. The Global Burden of Cancer 2013. JAMA Oncol 2015;1(4):505–727. DOI: 10.1001/jamaoncol.2015.0735. Erratum in: 2015;1(5):690.

3) Tanaka T, Ishigamori R. Understanding carcinogenesis for fighting oral cancer. *J Oncol.* 2011;2011:603740.

4) Gharat SA, Momin M, Bhavsar C. Oral squamous cell carcinoma: current treatment strategies and nanotechnology-based approaches for prevention and therapy. Crit Rev Ther Drug Carrier Syst 2016;33(4):363–400. DOI: 10.1615/CritRevTherDrugCarrierSyst.2016016272

5) Bishayee A, Sethi G. Bioactive natural products in cancer prevention and therapy: progress and promise. Semin Cancer Biol 2016;40–41:1–3. DOI: 10.1016/j.semcancer.2016.08.006

6) Gutiérrez J.M., Calvete J.J., Habib A.G., Harrison R.A., Williams D.J., Warrell D.A. Snakebite envenoming. *Nat. Rev. Dis. Prim.* 2017;3:17063. doi: 10.1038/nrdp.2017.63

7) Pal SK, Gomes A, Dasgupta SC, Gomes A (2002) Snake venom as therapeutic agents: from toxin to drug development. Indian J Exp Biol 40(12):1353–1358

8) Earl ST, Birrell GW, Wallis TP, St Pierre LD, Masci PP, de Jersey J, Gorman JJ, Lavin MF (2006) Post-translational modification ac- counts for the presence of varied forms of nerve growth factor in Australian elapid snake venoms. Proteomics 6(24):6554–6565. doi: 10.1002/pmic.200600263

9) Chan Y.S., Cheung R.C.F., Xia L., Wong J.H., Ng T.B., Chan W.Y. Snake venom toxins: Toxicity and medicinal applications. *Appl. Microbiol. Biotechnol.* 2016;100:6165–6181. doi: 10.1007/s00253-016-7610-9.

10) Essex H.E., Priestley J.T. Effect of rattlesnake venom on flexner-jobling's carcinoma in the white rat (*mus norvegicus albinus.*) *Proc. Soc. Exp. Biol. Med.* 1931;28:550–551. doi: 10.3181/00379727-28-5414.

11) J. K. S. Al-Safi, A. Bansal, M. Aarif, M. S. Z. Almahairah, G. Manoharan and F. J. Alotoum, "Assessment Based On IoT For Efficient Information Surveillance Regarding Harmful Strikes Upon Financial Collection," 2023 International Conference on Computer Communication and Informatics (ICCCI), Coimbatore, India, 2023, pp. 1-5, doi: 10.1109/ICCCI56745.2023.10128500.

12) Tidake, Vishal & Mazumdar, Nilanjan & Kumar, A. & Rao, B. & Fatma, Dr Gulnaz & Raj, I.. (2023). Sentiment Analysis of Movie Review using Hybrid Optimization with Convolutional Neural Network in English Language. 1668-1673. 10.1109/ICAIS56108.2023.10073750.

13) M. A. Tripathi, R. Tripathi, F. Effendy, G. Manoharan, M. John Paul and M. Aarif, "An In-Depth Analysis of the Role That ML and Big Data Play in Driving Digital Marketing's Paradigm Shift," 2023 International Conference on Computer Communication and Informatics (ICCCI), Coimbatore, India, 2023, pp. 1-6, doi: 10.1109/ICCCI56745.2023.10128357.

14) M. Lourens, A. Tamizhselvi, B. Goswami, J. Alanya-Beltran, M. Aarif and D. Gangodkar, "Database Management Difficulties in the Internet of Things," 2022 5th International Conference on Contemporary Computing and Informatics (IC3I), Uttar Pradesh, India, 2022, pp. 322-326, doi: 10.1109/IC3I56241.2022.10072614.

15) Abd Algani, Y. M., Caro, O. J. M., Bravo, L. M. R., Kaur, C., Al Ansari, M. S., & Bala, B. K. (2023). Leaf disease identification and classification using optimized deep learning. *Measurement: Sensors*, *25*, 100643.

16) Ratna, K. S., Daniel, C., Ram, A., Yadav, B. S. K., & Hemalatha, G. (2021). Analytical investigation of MR damper for vibration control: a review. *Journal of Applied Engineering Sciences*, *11*(1), 49-52.

17) Abd Algani, Y. M., Ritonga, M., Kiran Bala, B., Al Ansari, M. S., Badr, M., & Taloba, A. I. (2022). Machine learning in health condition check-up: An approach using Breiman's random forest algorithm. *Measurement: Sensors*, *23*, 100406. https://doi.org/10.1016/j.measen.2022.100406

18) Mourad, H. M., Kaur, D., & Aarif, M. (2020). Challenges Faced by Big Data and Its Orientation in the Field of Business Marketing. *International Journal of Mechanical and Production Engineering Research and Development (IJMPERD)*, *10*(3), 8091-8102.

19) Aarif, Mohd, and Ali Alalmai. "Importance of Effective Business Communication for promoting and developing Hospitality Industry in Saudi Arabia." *A case study of Gizan (Jazan)* (2019).

20) Mourad, H. M., Kaur, D., & Aarif, M. (2020). Challenges Faced by Big Data and Its Orientation in the Field of Business Marketing. *International Journal of Mechanical and Production Engineering Research and Development (IJMPERD)*, *10*(3), 8091-8102.

21) Kurotchkin T., Spies J. Effects of cobra venom on the Fujinami rat sarcoma. *Proc. Soc. Exp. Biol. Med.* 1935;32:1408–1410. doi: 10.3181/00379727-32-8111P.

22) Drueck C.J. Cdbra venom and opiates in the pain of cancer of the rectum. *Anesth. Analg.* 1942;21:41–45.

23) Liu CC, Yang H, Zhang LL, Zhang Q, Chen B, Wang Y (2014) Biotoxins for cancer therapy. Asian Pac J Cancer Prev 15(12):4753–4758

24) Li L, Huang J, Lin Y. Snake Venoms in Cancer Therapy: Past, Present and Future. Toxins (Basel). 2018 Aug 29;10(9):346. doi: 10.3390/toxins10090346. PMID: 30158426; PMCID: PMC6162746.

25) Yen CY, Liang SS, Han LY, Chou HL, Chou CK, Lin SR, Chiu CC. Cardiotoxin III inhibits proliferation and migration of oral cancer cells through MAPK and MMP signaling. ScientificWorldJournal. 2013 Apr 8;2013:650946. doi: 10.1155/2013/650946. PMID: 23710144; PMCID: PMC3654281.

26) Chiu CC, Lin KL, Chien CM, Chang LS, Lin SR. Effects of cardiotoxin III on NF-κB function, proliferation, and apoptosis in human breast MCF-7 cancer cells. *Oncology Research.* 2009;17(7):311–321.

27) Yang SH, Chien CM, Chang LS, Lin SR. Involvement of c-jun N-terminal kinase in G2/M arrest and caspase-mediated apoptosis induced by cardiotoxin III (Naja naja atra) in K562 leukemia cells. *Toxicon.* 2007;49(7):966–974.

28) Tsai CH, Yang SH, Chien CM, et al. Mechanisms of cardiotoxin III-induced apoptosis in human colorectal cancer Colo205 Cells. *Clinical and Experimental Pharmacology and Physiology.* 2006;33(3):177–182.

29) Chien CM, Lin KL, Su JC, Chang LS, Lin SR. Inactivation of epidermal growth factor receptor and downstream pathways in oral squamous cell carcinoma Ca9-22 cells by cardiotoxin III from Naja naja atra. *Journal of Natural Products.* 2009;72(10):1735–1740.

30) Chien CM, Chang SY, Lin KL, Chiu CC, Chang LS, Lin SR. Taiwan cobra cardiotoxin III inhibits Src kinase leading to apoptosis and cell cycle arrest of oral squamous cell carcinoma Ca9-22 cells. *Toxicon.* 2010;56(4):508–520.

31) da Rocha RG, Santos EMS, Tanaka-Azevedo AM, Serino-Silva C, Souza MG, Gomes ESB, Guimarães FAD, Silveira LH, Santos SHS, de Paula AMB, Gomez RS, Guimarães ALS, Farias LC. The antineoplastic potential of crotoxin isolated from Crotalus durissus terrificus snake venom on oral squamous cell carcinoma. Toxicon. 2023 Jan 1;221:106965. doi: 10.1016/j.toxicon.2022.106965. Epub 2022 Nov 9. PMID: 36370827.

32) Chai L, Huang T, Wang Z, et al. AAVC-I promotes apoptosis of human oral squamous cell carcinoma through the mitochondrial pathway. Int J Clin Exp Pathol 2019;12(10):3968–3974. PMID: 31933792; PMCID: PMC6949759.

33) Bordon KCF, Cologna CT, Fornari-Baldo EC, et al. From animal poisons and venoms to medicines: achievements, challenges and perspectives in drug discovery. Front Pharmacol 2020;11:1132. DOI: 10.3389/fphar.2020.01132. PMID: 32848750; PMCID: PMC7396678.

34) King, G. F. (2011). Venoms as a platform for human drugs: translating toxins into therapeutics. *Expert Opin. Biol. Ther.* 11 (11), 1469–1484. doi: 10.1517/14712598.2011.621940

35) Siu D. Natural products and their role in cancer therapy. Med Oncol 2011;28(3):888–900. DOI: 10.1007/s12032-010-9528-x

36) Kashwani R, Sawhney H, Dentistry and metaverse: A deep dive into potential of blockchain, NFTs, and crypto in healthcare. Int Dent J Stud Res 2023;11(3):94-98 DOI: https://doi.org/10.18231/j.idjsr.2023.021