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# Exploring the mechanism and component basis of *Naja naja* (Snake) venom for the treatment of cancer by network pharmacology and molecular docking techniques

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## ABSTRACT

**Background:** Numerous experimental models proved the protective effect of Naja naja snake venom on various cancers. However, their mechanism of action is not known. In the present study, network pharmacology and molecular docking technology were performed to analyse the molecular mechanism of components of Naja naja for the treatment of cancer.

**Methods:** The venom peptide structures were obtained from pubchem databases and RCSB PDB. The targets of snake venom peptides were obtained from literature survey. The protein-protein interaction network was done using the STRING webtool and ShinyGo 0.80 software. The binding affinity between potential targets and active compounds was evaluated by molecular docking using Autodock vina.

**Result:** By analysing string programme, the highest degree of protein interaction was found between AChE and metelloprotease and the proteins of antioxidant, apoptotic, clock and inflammation, implying that these enzymes may exert its anti-cancer action through the regulation of the above said pathways.

**Conclusion:** It is difficult to find the therapeutic target of all snake peptides/protein, because the structures for most of them are not available. So, in this study, we explored the molecular target of few snake venom peptides/proteins using string analysis and studied the structure-function relationships of snake venom peptides. This *in silico* experiment will paved a way for designing preclinical and clinical experiments.

## INTRODUCTION

The Global Cancer Observatory (GLOBOCAN) demonstrated a incidence of 19.3 million cancer cases globally in 2020 and the United States of America, China and India are ranking first, second and third among all other countries (Sathishkumar et al., 2025; Sung et al., 2021). The majority of cancers incidences (90-95%), are brought on by lifestyle and environmental influences. Genetic inheritance is responsible for the remaining 5-10% cases (Anand *et al.*, 2008). Cancer is an uncontrolled cell proliferation with a wide range of phenotypic manifestations, from mild to lethal. Cancer may develop from a number of factors, such as DNA damage or mutation, any consequence of that DNA, or inability of the regulatory and repressor systems to function along the cycle (Calcaterra *et al.*, 2020).

In general, it is recognised that the rise in ROS in malignant cells plays a significant effect in the development and progression of cancer. The increased levels of ROS leads to increased rate of metabolism, mitochondrial dysfunction, activity of peroxisome, cyclooxygenases, lipooxygenases and thymidine phosphorylase (Storz 2005). ROS-mediated signaling pathways are constantly enhanced in several cancers, where they contribute in cell division, differentiation, proliferation, glucose metabolism, protein synthesis, inflammation and cell survival (Storz 2005). Numerous factors including medications and radiation, have been found in studies to interfere with the circadian clock (Rohling et al., 2011). Circadian desynchrony is linked to an increased risk of illnesses that have an impact on all facets of human health, such as obesity, depression, metabolic diseases and cancer (Nernpermpisooth et al., 2015). Kerr *et al.* (1972) indicated that apoptosis was linked with the elimination of cancerous cells, development of tumours and hyperplasia. Diminished or resistance to apoptosis plays a key role during the process of carcinogenesis. Cancer cell can reduce or increase apoptotic resistance. It can be divided into following categories which includes 1) changes in the ratio of pro and anti-apoptotic markers 2) decreased function of caspase and 3) diminished signalling of death receptor.

Proteins play a key role in various biological activities like transport and signalling molecules, receptor function, regulators of gene expression, enzymatic and immunological reactions. Apart from these functions, it involves in the replication, transcription and translation processes. These functions are regulated by precise protein-protein interactions (PPIs) (Peng *et al.*, 2017). PPIs in cells are collectively called as "interactome". The PPI has a significant effect in both normal and diseased processes, such as differentiation and proliferation, growth, interaction and survival of cells (Nero *et al.*, 2014). Hence, the alterations in PPIs are linked with diabetes, tumors,

atherosclerosis, microbial infections, kidney disorders and neurological diseases (White *et al.*, 2008). As the drug molecules including peptide or protein molecules mainly target the receptors, enzymes and ion channels, for their therapeutic action, the PPIs are needed to study for the development of novel and potential therapeutic targets (Nevola and Giralt 2015). The PPI modulators mainly divided into three types. The first type belongs to small molecule modulators. The small molecule modulator may not face the difficulties in ADME processes, because of their smaller size (Sheng *et al.*, 2015). But they do not have large surface area for making huge number of hydrophobic contacts. The second type belongs to antibody. Although monoclonal antibodies are used as potent therapeutic agents, due to their hefty molecular weight and potential to trigger side effects linked with the immune reactions, the use of them is restricted. The third one belongs to peptides. The peptides or proteins are able to bind strongly with proteins and retain their functions. As compared to other two types, the molecular weight of peptides are intermediate.

The snake venom is rich in several active proteins such as acetylcholinesterases, serine proteases, metalloproteases, L-amino acid oxidases, phospholipases A2 etc and peptides like disintegrins, neurotoxin, cardiotoxin, natriuretic peptide, crotamine, sarafotoxin, waglerin etc with potent therapeutic activities. The present study is designed to explore the interactions between the proteins (acetylcholinesterases, serine and metalloproteases, L-amino acid oxidases and phospholipases A2) and peptides (3FTX, neurotoxin, cardiotoxin, cytotoxin and disintegrin) of snake venom on oxidative stress (SOD, catalase, GPx, GSH and GST), clock genes (*per*, *tim*, *cry 1*, *Bmal 1* and *clock*), apoptosis (Bax, Bcl2, cytochrome C, caspases 3, 6, 8 and 9) and inflammation (Interleukins-1β,-6 and -10, TNF-α, NF κB, COX-2 and iNOS) related proteins. Moreover, to identify the targets of these molecules, a network was constructed by using the STRING database. Within this network, common targets of these peptides/proteins were identified, followed by an exploration of the interactions of them in relation to oxidative stress, clock genes, inflammation and apoptosis using the STRING database.

**MATERIALS AND METHODS**

The venom peptide structures were obtained from the PubChem databases and RCSB PDB. The targets of snake venom peptides were sourced from literature survey. The PPI network studies were done by the STRING webtool. The pathway enrichment analysis was done using ShinyGo 0.80 software. The binding affinity found between pathological processes and active compounds were studied using molecular docking (Autodock vina).

**RESULTS**

Fig. 1A, B, and Table 1 indicated that a few proteins of apoptotic (caspase 3, caspase 6, and caspase 9) and inflammatory (IL-1β, -6, COX-2, TNF-α, and NF-κB) pathways interacted with the acetylcholinesterase enzyme.

Fig. 2A, B, C, and Table 2 demonstrated that the antioxidant (GPx) and apoptotic (Bcl2) markers interacted with the protein serine protease present in the venom. A few proteins of the inflammatory pathway (TNF-α and IL-10) also interacted with the serine protease enzyme.

Fig. 3A, B, and Table 3 showed that there is no interaction of metalloprotease with antioxidants (SOD, catalase, GPx, GSH, GST) and clock genes (*Per*, *tim*, *cry 1*, *BMAL1*, *clock*). Apoptotic markers (BCL2, Bax, Cyto-C, Caspases 3 and 9) showed interaction with the protein metalloprotease present in the venom. A few proteins of the inflammatory pathway (IL-1β and -6, iNOS, and TNF-α) interacted with the metalloprotease enzyme.

Fig.4 and Table 4 specified that the inflammatory markers such as IL-6, TNF-α, and IL-10 interacted with cytotoxin. Other antioxidants (SOD, catalase, GPx, GSH, GST), apoptotic markers (Bax, Bcl2, cytochrome C, caspases 3, 6, 8, and 9), and clock genes showed no interaction.

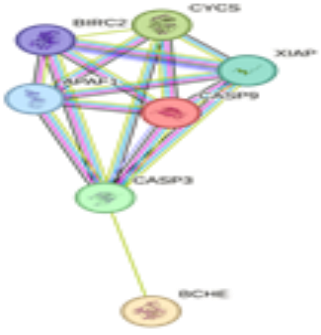
Fig.5 and Table 5 showed that a clock protein (per) only interacted with disintegrin. Other inflammatory markers (IL-1β and -6, cox-2, TNF- α, NF-κB, IL-10,), antioxidants (SOD, catalase, GPx, GSH, GST), clock genes (*per*, *tim*, *cry 1*, *BMAL1*, *clock*) and apoptotic markers (Bax, Bcl-2, cytochrome C, caspases 3, 6, 8 and 9) showed no interaction.

The string analysis indicated that there is no direct interaction between phospholipase, 3FTx, neurotoxin, cardiotoxin, and L-amino acid oxidase with antioxidant, inflammatory, apoptotic, and clock gene markers. So, they are not mentioned in the results section.

**Fig. 1A**

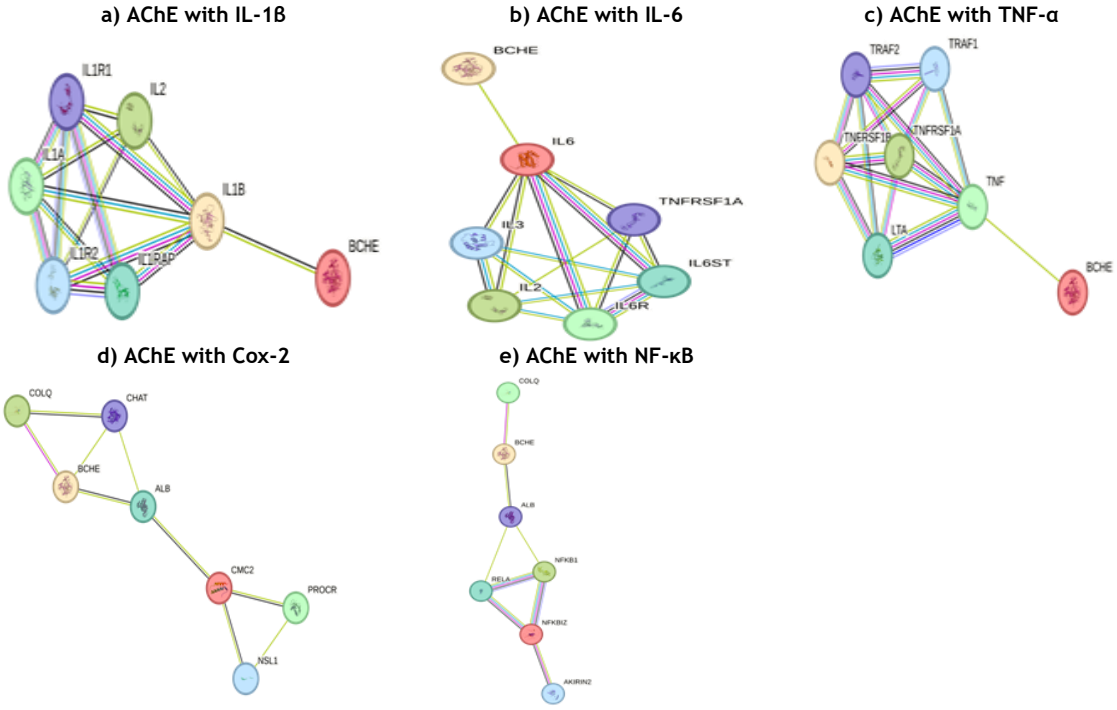
**a) AChE with caspase 3**


**b) AChE with caspase 6**

**c) AChE with caspase 9**

Fig. 1B




Fig, 1. Protein-protein interaction (PPI) network of Acetylcholine esterase with (A) apoptotic markers and (B) inflammatory markers) by STRING program analysis.
**Table 1:** KEGG analysis of the acetylcholine esterase (butylcholine esterase) on antioxidants, apoptosis, clock genes and inflammation pathways.

| Protein in the venom of *Naja naja* | Process/ Pathway | Marker proteins | FDR |
|---|---|---|---|
| Acetylcholine esterase | Antioxidants | - | - |
| | Apoptosis | Caspase 3, Caspase 6, Caspase 9 | 9.23e-11, 1.67e-05, 5.63e-14 |
| | Clock genes | - | - |
| | Inflammation | IL-1ß, IL-6, TNF-α, COX-2, NFκ- B | 0.0083, 0.0044, 9.01e-11, 3.61e-05, 0.0153 |

**Fig. 2A**
**a) SP with GPx**

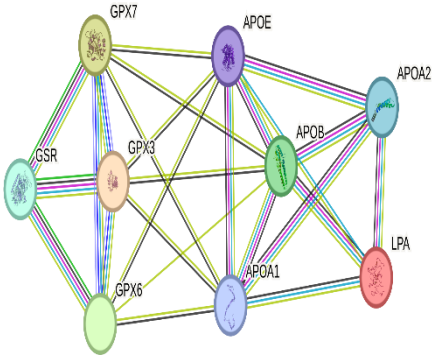



**Fig.2B.**
**b) SP with BCl-2**

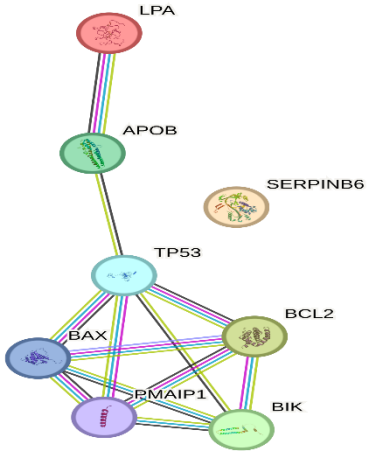



**Fig. 2C**

**a) SP with TNF- α** **b) SP with IL- 10**

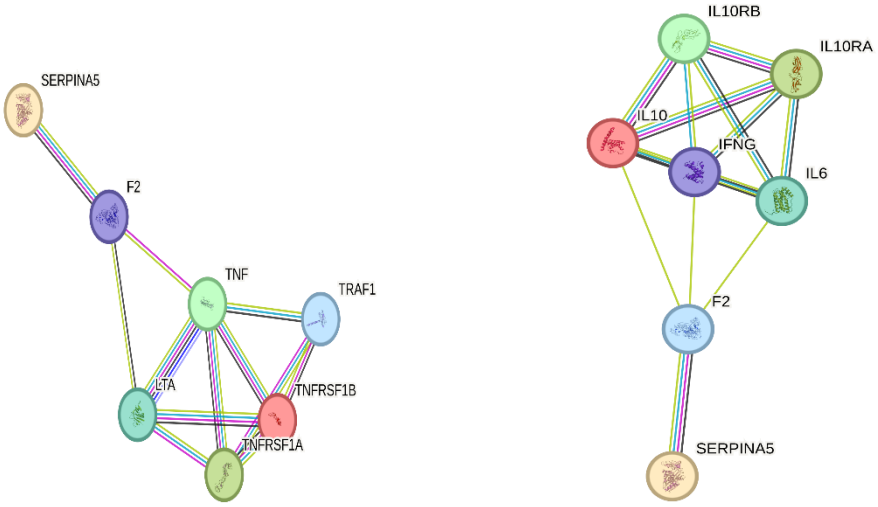



Fig. 2. PPI network of serine protease with oxidative stress marker (A), apoptotic markers (B) and inflammatory markers (C) by STRING program analysis.

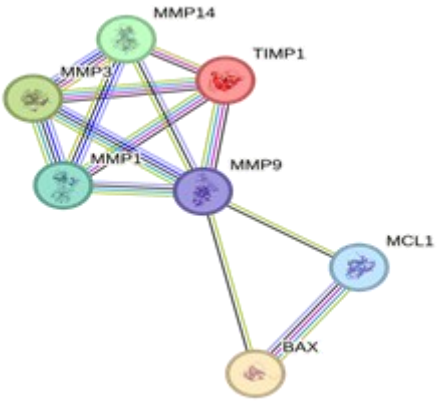
**Table 2.** KEGG analysis of the serine protease on antioxidants, apoptosis, clock genes and inflammation pathways.

| Protein in the venom of *Naja naja* | Process/Pathway | Marker proteins | FDR |
|---|---|---|---|
| Serine protease | Antioxidants | GPx | 1.22e-06 |
| | Apoptosis | Bcl2 | 1.33e-05 |

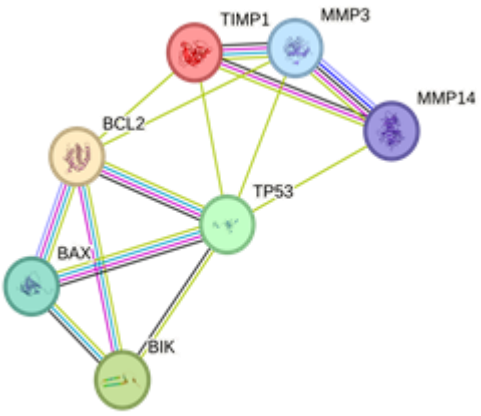
| | | | |
|---|---|---|---|
| | Clock genes | - | |
| | Inflammation | TNF α, IL-10 | 4.53e-08, 4.32e-05 |

**Fig. 3A**

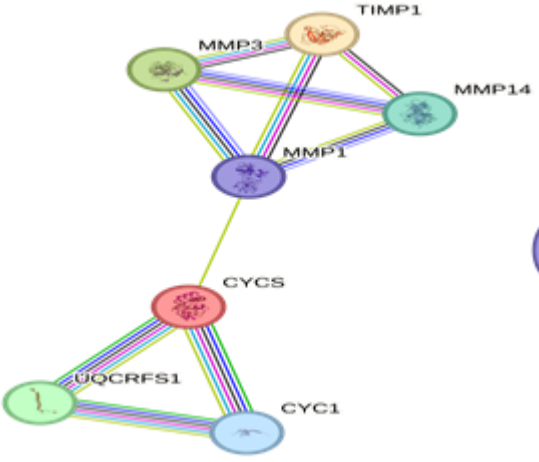
**a) MP with Bax**



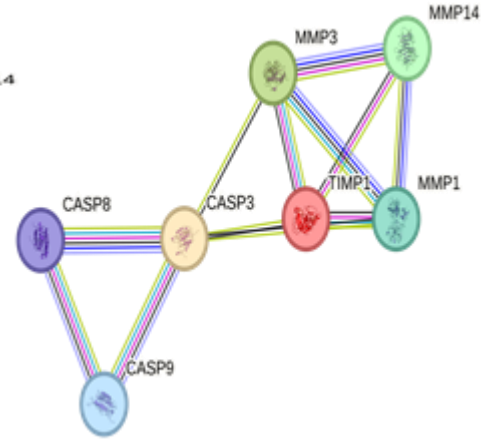

**b) MP with $BCl_2$**




**c) MP with Cytochrome C**




**d) MP with caspase 3**




**e) MP with caspase 9**

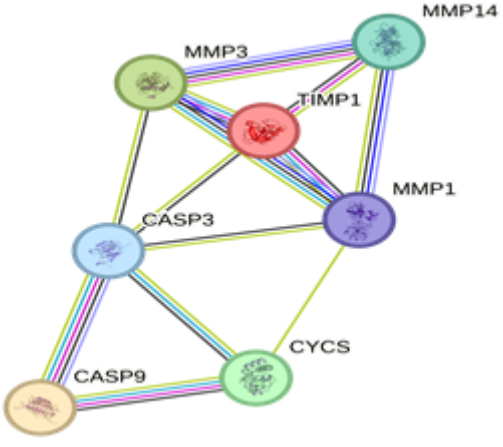



**Figure 3B.**

**a) MP with IL-1β**

**b) MP with IL-6**

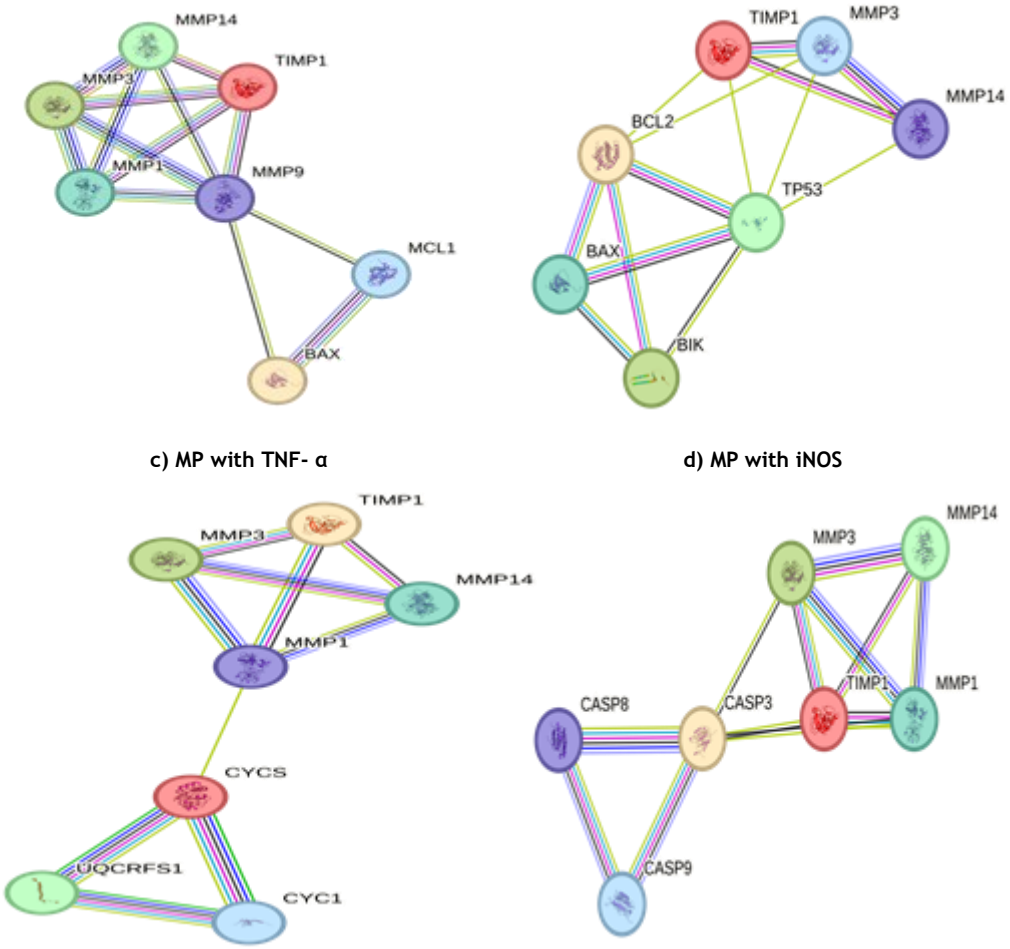



Fig. 3. Protein-protein interaction (PPI) network of metalloprotease with apoptotic (A) and inflammatory (B) markers by STRING program analysis.

**Table 3** Table 3 KEGG analysis of the metalloprotease on antioxidants, apoptosis, clock genes and inflammation pathways.

| Protein in the venom of *Naja naja* | Process/ Pathway | Marker proteins | FDR |
|---|---|---|---|
| Metalloprotease | Antioxidants | - | |
| | Apoptosis | BCl-2<br>Bax<br>Cyto C<br>Caspase 9<br>Caspase 3 | 0.0011<br>0.0321<br>2.01e-07, 5.01e-05<br>1.67e-05 |
| | Clock genes | - | |
| | Inflammation | IL-1β,<br>IL-6,<br>TNF-α,<br>iNOS | 0.00012, 0.0053,<br>0.0011,<br>0.0013 |

**Fig. 4.**

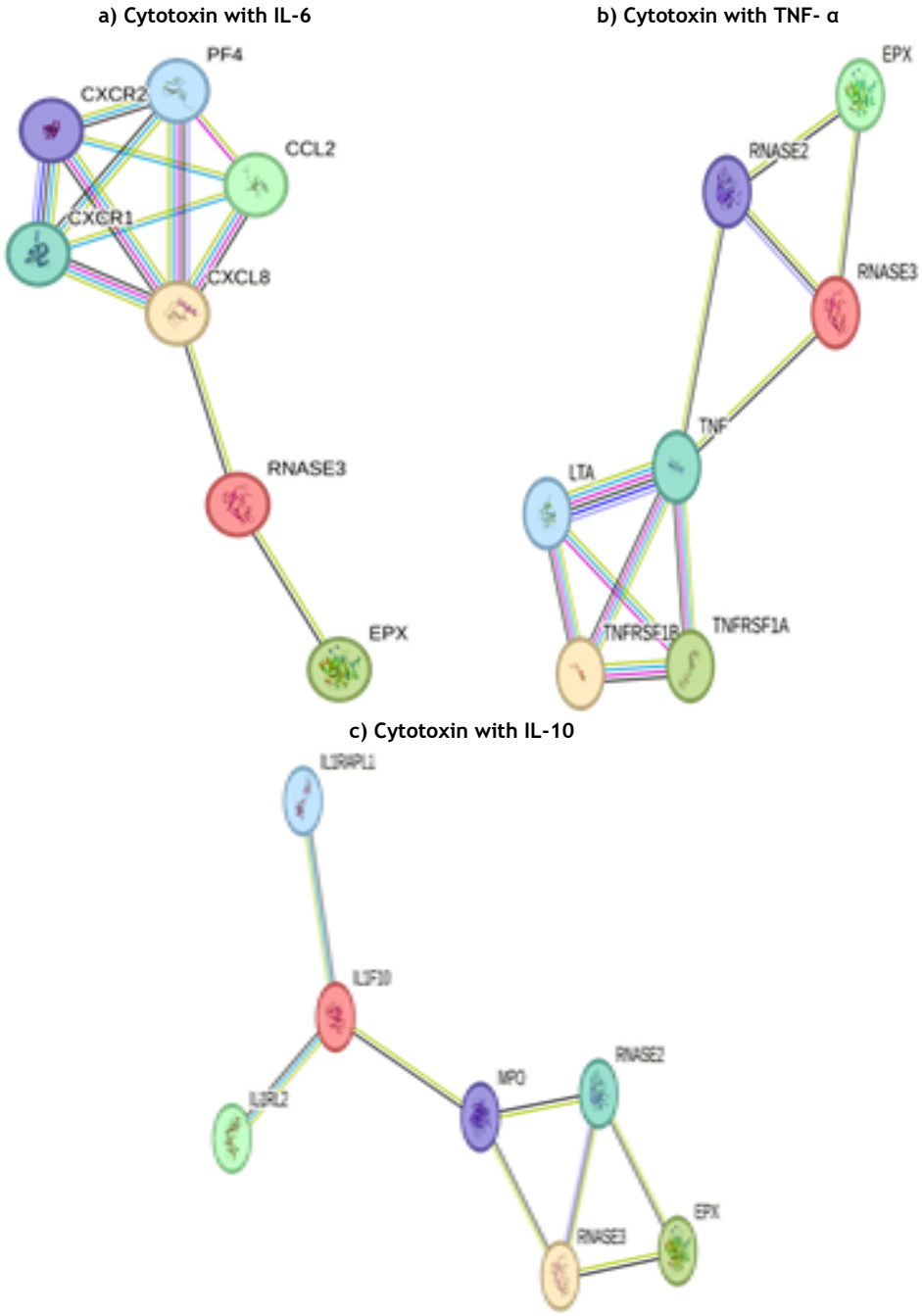



Fig. 4 Protein-protein interaction (PPI) network of cytotoxin with inflammatory markers by STRING program analysis.

**Table 4:** KEGG analysis of the cytotoxin on antioxidants, apoptosis, clock genes and inflammation pathways.

Fig.5

| Protein in the venom of *Naja naja* | Process/ Pathway | Marker proteins | FDR |
|---|---|---|---|
| Cytotoxin | Antioxidants | - | |
| | Apoptosis | - | |
| | Clock genes | - | |
| | Inflammation | IL-6, TNF α, IL-10 | 0.0153, 7.26e-06, 0.0147 |

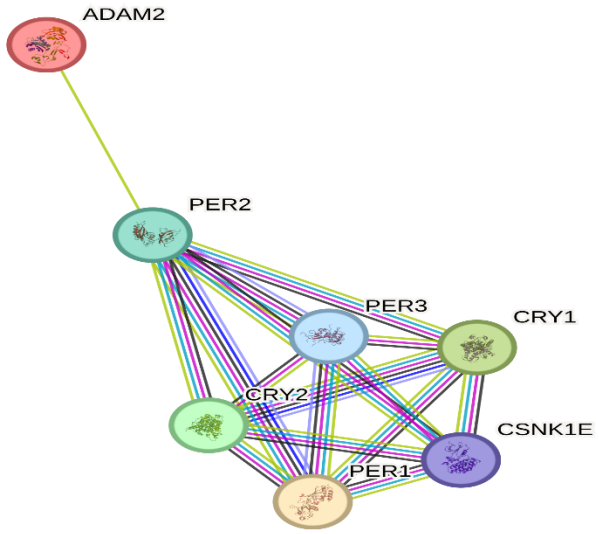



Fig. 5. Protein-protein interaction (PPI) network of disintegrin with Clock gene Per by STRING program analysis.

**Table 5.** KEGG analysis of the disintegrin on antioxidants, apoptosis, clock genes and inflammation pathways.

| Protein in the venom of *Naja naja* | Process/Pathway | Marker proteins | FDR |
|---|---|---|---|
| Disintegrin | Antioxidants | - | |
| | Apoptosis | - | |
| | Clock genes | *Per* | 5.63e-14 |
| | Inflammation | - | |

## DISCUSSION

Peptides are considering as the most interesting constituent of snake venom. Although they are considered as poisonous, their usage in appropriate concentrations or genetically altered, may lead them to be used directly as pharmacological drugs or drug leads (Uzair *et al.*, 2018). Due to their diversity, specific therapeutic properties and high selectivity and affinity towards their receptors, they are having great value in the treatment of cancer (Dutertre *et al.*, 2017). These peptides are highly stable because of their ability to subsist in the punitive proteolytic environment of the gland itself. As the key therapeutic target of these peptides and proteins in the destruction of cancer is not well understood, the present experiment use bioinformatics for finding the key protein targets and their downstream signal processing pathways. By analysing string programme, the best degree of protein interaction was found between AChE and the proteins involved in antioxidant, apoptotic, clock and inflammation function, implying that the enzyme may exert its anti-cancer action through the regulation of the abovesaid pathways.

Metalloproteases are major component of several crotalid and viperid snakes. They induced hemorrhage by altering blood coagulation process or binding with various constituents of extracellular matrix. In our study, they are reported to have interactions with apoptotic and inflammatory pathways. Gabriel *et al.* (2012) indicated that metalloproteinase from *Bothrops leucurus* induced apoptosis and cellular morphologic changes and also inhibits cell proliferation, which is corroborate with our results. Disintegrins are the nonenzymatic, nontoxic and small molecules with molecular weight of 5–10 kDa Arginyl-glycyl-aspartic acid-containing peptides that are found in snake venoms or synthetics. These proteins showed interaction with per protein of clock genes. Various members of disintegrin family such as rhodostomin, triflavin, eritostatin, trigramin, salmosin, contortrostatin, albolabrin, obtustatin and echistatin were reported to have anti-tumorigenic activity (Calderon *et al.*, 2014).

In our study, the cytotoxin protein interact with the inflammatory proteins. Attarde and Pandit (2020) investigated the antitumoral effect of purified cytotoxin and cardiotoxin obtained from the *Naja naja atra* snake. Other experiments involving cytotoxin P4 (*Naja naja atra*), showed anatomical alterations in leukemia cells particularly in mitochondria in both the *in vitro* and *in vivo* studies (Chaim-Matyas *et al.*, 1991). Various cytotoxins such as CT1 & CT2 (*N. oxiana*), CT3 (*N. kaouthia*) and CT1 (*N. haje*) are reported to induce histopathological changes in promyelocytic leukemia (HL60) and lung adenocarcinoma (A549) cells (Feofanov *et al.*, 2005).

The results of our study indicated the interaction of serine protease with antioxidant (GPx), apoptotic (BCl-2) and inflammatory (TNF-α & IL-10) proteins. Serineproteases are enzymes that are present in lower microorganisms, plants and animals. These enzymes play a key role in digestion, complement activation, cell differentiation, etc. Few studies were carried regarding their anti-tumor effect and mechanisms of action. Markland (1986) studied the role of crotalase obtained from *Crotalus adamanteus*, a serine protease on the growth of B16 melanoma cells in *in vitro* conditions and also in C57BL/6 mice (*in vivo*). This enzyme was able to retard the growth of tumor cells in lab conditions and also not showed any cytotoxic or cytostatic alteration in the animals and further enhanced viability of the animals.

Although several advanced and integral techniques are available to isolate and characterize the snake venom peptides, only few structures of them are available in the literature. The protein structure of important snake venom peptide/proteins such as L-Amino acid oxidase, were not available. It is difficult to find the therapeutic target of all snake peptides/protein. So, in the experiment, we explored the molecular target of few snake venom peptides/proteins using string analysis and studied the structure-function mechanisms of snake venom peptides. This *in silico* experiment paved a way for the designing our *in vitro* experiments.

## CONCLUSION

In our study, we investigated the interactions between the peptide/protein of interest such as Acetylcholine esterase, L-Aminoacid oxidase, Phospholipase A, 3Finger toxins, metalloproteinases, serine proteinase, cardiotoxin, neurotoxin, cytotoxin and disintegrins and its targets in the regulation of cellular homeostasis, a network was constructed using the STRING database. Within this network, common targets of these peptides were identified, followed by an exploration of the interactions in relation to antioxidant activity, apoptotic

activity, clock genes activity and inflammation using the STRING database.

**ACKNOWLEDGEMENT**

Nil.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest regarding this manuscript.

## REFERENCES


- Anand, P., Kunnumakkara, A.B., Sundaram, C., Harikumar, K.B., Tharakan, S.T., Lai, O.S., Sung, B., Aggarwal, B.B. 2008. Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.*, 25 (9): 2097–116.
- Attarde, S.S., Pandit, S.V. 2020. Anticancer potential of nanogold conjugated toxin GNP-NN-32 from Naja naja venom. *J Venom Anim Toxins Incl Trop Dis.*, 26: e20190047.
- Calcaterra, I., Iannuzzo, G., Dell'Aquila, F., Di Minno, M.N.D. 2020. Pathophysiological role of synovitis in hemophilic arthropathy development: A Two-hit hypothesis. *Front Physiol.*, 11:541.
- Calderon, L.A., Sobrinho, J.C., Zaqueo, K.D., de Moura, A.A., Grabner, A.N., Mazzi, M.V., Marcussi, S., Nomizo, A., Fernandes, C.F., Zuliani, J.P., Carvalho, B.M., da Silva, S.L., Stabeli, R.G., Soares, A.M. 2014. Antitumoral activity of snake venom proteins: new trends in cancer therapy. *Biomed Res Int.*, 203639.
- Chaim-Matyas, A., Borkow, G., Ovadia, M. 1991. Isolation and characterization of a cytotoxin P4 from the venom of *Naja nigricollis* preferentially active on tumor cells. *Biochem Int.*, 24(3): 415–421.
- Dutertre, S., Nicke, A., Tsetlin, V.I. 2017. Nicotinic acetylcholine receptor inhibitors derived from snake and snail venoms. *Neuropharmacology.*, 127: 196–223.
- Feofanov, A.V., Sharonov, G.V., Astapova, M.V., Rodionov, D.I., Utkin, Y.N., Arseniev, A.S. 2005. Cancer cell injury by cytotoxins from cobra venom is mediated through lysosomal damage. *Biochem. J.*, 390(1):11–18.
- Gabriel, L.M., Sanchez, E.F., Silva, S.G., Santos, R.G. 2012. Tumor cytotoxicity of leucurolysin-B, a P-III snake venom metalloproteinase from Bothrops leucurus. *J. Venom. Anim. Toxins Incl. Trop. Dis.*, 18(1): 24–33.
- Kerr, J.F.R., Wyllie, A.H., Currie, A.R. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer.*, 26: 239–257.
- Markland, F.S, Jr. 1986. Antitumor action of crotalase, a defibrinogenating snake venom enzyme. *Semin. Thromb. Hemost.*, 12(4):284–290.
- Nernpermpisooth, N., Qiu, S., Mintz, J.D., Suvitayavat, W., Thirawarapan, S., Rudic, D.R., Fulton, D.J., Stepp, D.W. 2015. Obesity alters the peripheral circadian clock in the aorta and microcirculation. *Microcirculation.*, 22(4):257–266.
- Nero, T.L., Morton, C.J., Holien, J.K., Wielens, J., Parker, M.W. 2014. Oncogenic protein interfaces: small molecules, big challenges. *Nat. Rev. Cancer.*, 14, 248–262.
- Nevola. L. and Giralt E. 2015. Modulating protein-protein interactions: the potential of peptides. *Chem. Commun.*, 51: 3302–3315.
- Peng, X., Wang, J., Peng, W., Wu, F., Pan, Y. 2017. Protein–protein interactions: detection, reliability assessment and applications. *Brief. Bioinformatics.*,18(5), 798–819.
- Rohling, J.H., vanderLeest, H.T., Michel, S., Vansteensel, M.J., Meijer, J.H. 2011. Phase resetting of the mammalian circadian clock relies on a rapid shift of a small population of pacemaker neurons. *PLoS One.*,6(9): e25437.
- Sathishkumar, K., Chaturvedi, M., Das, P., Stephen, S., Mathur, P. 2022. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. *Indian J Med Res.*,156(4&5):598-607.
- Sheng, C., Dong, G., Miao, Z., Zhang, W., Wang, W. 2015. State-of-the-art strategies for targeting protein-protein interactions by small-molecule inhibitors. *Chem. Soc. Rev.* 44: 8238–8259.
- Storz, P. 2005. Reactive oxygen species in tumor progression. *Front Biosci.*, 10:1881–96.
- Sung, H., Ferlay, J., Siegal, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J Clin.*, 71(3): 209-249.
- Uzair, B., Atlas, N., Malik, S.B., Jamil, N., Salaam, T.O., Rehman, M.U., Khan, B.A. 2018. Snake venom as an effective tool against colorectal cancer. *Protein Pept. Lett.*, 25:626–632.
- White, A.W., Westwell, A.D., Brahemi, G. 2008. Protein-protein interactions as targets for small-molecule therapeutics in cancer. *Expert Rev. Mol. Med.*, 10: e8.