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Review Article

Anticancer potential of animal venoms and toxins

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Anticancer drug development from natural resources are ventured throughout the world. Animal venoms and toxins a potential bio resource and a therapeutic tool were known to man for centuries through folk and traditional knowledge. The biodiversity of venoms and toxins made it a unique source of leads and structural templates from which new therapeutic agents may be developed. Venoms of several animal species (snake, scorpion, toad, frog etc) and their active components (protein and non protein toxins, peptides, enzymes, etc) have shown therapeutic potential against cancer. In the present review, the anticancer potential of venoms and toxins from snakes, scorpions, toads and frogs has been discussed. Some of these molecules are in the clinical trials and may find their way towards anticancer drug development in the near future. The implications of combination therapy of natural products in cancer have been discussed.

Keywords: Anticancer activity, Combination therapy, Toxins, Venoms

Venoms and toxins not only caused global morbidity and mortality, but are also used as potential probe for illuminating multifaceted biological processes. By definition, venoms are the secretion of venomous animals, which are synthesized and stored in specific areas of their body i.e., venom glands. The animals use venoms for defense or to immobilize the prey. Most of the venoms are complex mixture of a number of proteins, peptides, enzymes, toxins and non protein inclusions. Venom composition and chemistry varies among species, age, sex and different geographic regions. Toxins, occurring in venoms and poisons of venomous animals, are chemically pure toxic substances with more or less specific actions on biological systems. The biodiversity and specificity of venoms and toxins make them a unique source of leads and structural templates from which new therapeutic agents are being developed.

Medicinal application of venoms and toxins has been mentioned in Ayurvedic, Unani, Chinese and Homeopathic system of medicines. For example, the ancient Indian physician Sushruta (7th century B.C.) used snake venom to prolong life. Charaka had the opinion that in case of "Udra Roga" (disease of gastrointestinal tract), which was uncontrollable by other medicinal measures, snake venom was very useful. In "Charak Samhita" and "Vagbata" cobra venom had been said to be useful in "Dushydara" and "Jalodara" (ascites). Venom of spider, bee, and snakes are routinely used in homeopathic medicine[1]. For more than 2000 years, *Buthus martensii* Karsch venom was used to treat different ailments as described in Chinese traditional medical practice. In ancient Gallenical preparation, known to the Chinese as Chan Su (Senso to the Japanese), toad and frog skin extracts have been used for treating various diseases. Chinese toad skins, soaked in wine, are used for the treatment of leukemia[2]. Use of animal venoms and toxins in ethnic folk medicine are the basis of modern research.

Venoms and toxins have found a niche in the pharmaceutical market. Several isolated toxins with a known mode of action have practical applications as pharmaceutical agents, diagnostic reagents or preparative tools[3]. Chinese medical companies and traditional drug stores have marketed amphibian parts like dried oviducts of the frog *Rana chensinensis* and

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toad (Bufonid) skin[4]. Captopril, the inhibitor of the angiotensin 1 converting enzyme isolated from brazilian snake is used in the treatment of lung cancer[5].

Cancer, despite the all out efforts from developed countries still causes one in five deaths. Surgery, chemotherapy, and radiotherapy provide inadequate protection and instead, affect normal cells along with the cancer cells. The search for cancer cure from natural product (plants and animals) has been practiced for over a century and the use of purified chemicals to treat cancer still continues. From 1940s to 2007, of the 155 new cytotoxic molecules developed, 47% are actually either natural product or directly derived from them[6]. Several studies have been undertaken during last three decades to find the anticancer property of venoms and toxins. These lead to the discovery of several promising molecule having anticancer activity, some of which are in clinical trial and may emerged to be a future drug in cancer therapy. The present communication is an effort to enlighten the anticancer activity found in the venoms and toxins of reptiles (snake), arthropods (scorpion) and amphibians (frog and toad) and their future prospects.

Anticancer activity of snake venom/toxin(s)

Claude Bernard, the father of Physiology, was the first to realize that the physiologically active components of snake venom may have therapeutic potentials. Use of venom in the treatment of cancer in laboratory animals was first reported by Calmette[7]. Elapid, Crotalid and Viperid venoms were subjected to a comparative screening for *in vivo* and *in vitro* cytotoxicity towards B16F10 melanoma and chondrosarcoma cell lines. It was found that elapid venoms possess considerably higher cytotoxic activity than that of Viperid or Crotalid venoms. Some Viperidae and all Crotalidae venoms examined caused the cells to become rounded, without losing their original volume, and to form aggregates[8]. Venoms from the snake family Elapidae, Crotalidae and Viperidae but not Hydrophidae caused lysis of Yoshida sarcoma cells[9]. Venoms from two Viperidae species (*Bothrops jararaca* and *Crotalus durissus terrificus*) acted directly on tumor cells. Their antitumour activity may be due to the indirect phenomenon of inflammatory response mediated by IL2, IL8 and TNF-α[10]. The anticarcinogenic activities of Indian monocellate cobra and Russell's viper venom were studied in carcinoma, sarcoma and leukemia models. Under *in vivo* experiments, it was found that the sub-lethal doses of the Indian Elapidae (monocellate cobra) and Viperidae (Russell's viper) venom caused cytotoxicity on EAC cells; it increased the lifespan of EAC cell containing mice and reinforced its antioxidant system[11]. Similarly, antitumour activity of Hydrophidae (*Lapemis curtus*) venom was also established against EAC in Swiss albino mice *in vivo* and HeLa, Hep2 tumor cell cultures *in vitro*[12].

The emphasis of research undertaken during past four decades have been on isolation and characterization of snake venom cytotoxins[13-15]. Cytotoxins isolated from different venom sources exhibited various physiological effects, such as modulation of the activity of membrane enzymes, depolarization of excitable membranes, inhibition of platelet aggregation, cardiac arrest, hemolysis and cytotoxicity[16]. Cytotoxins or cardiotoxins are a group of polypeptides (60-70 amino acid residues) present in the snakes of elapid family having a wide variety of pharmacological actions such as haemolysis, depolarisation of muscles. Cardiotoxin 3 (CTX 3), a basic polypeptide of 60 amino acid residues with anticancer activity, was isolated from *Naja naja atra* venom[17]. CTX 3 inhibited the growth of K562 cells in a dose and time-dependent manner. It displayed several features of apoptosis including apoptotic body formation, increase in sub G1 population, DNA fragmentation, and poly (ADP-ribose) polymerase (PARP) cleavage. CTX 3 also induced apoptosis of K562 cells through a mitochondria and caspase-dependant mechanism[18]. CTX 3 induced apoptosis was triggered by Ca^{2+} influx as evidenced by rapid and persistent increase in cytosolic Ca^{2+} concentration after CTX 3 treatment to K562 cells. The apoptotic signal like caspase-12 and c-Jun N-terminal kinase (JNK) activation induced by CTX 3 were inhibited by intracellular Ca^{2+}chelator[19].

A heat stable 7.2 kDa protein toxin (drCT-I) was isolated and purified from the Indian russell's viper (*Daboia russelli russelli*) venom. Its first 20 N-terminal amino acids sequence showed homology to cytotoxins isolated from Naja venom[20]. drCT-I posses antiproliferative, cytotoxic and apoptotic activity[21]. From the Indian russell's viper (*Daboia russelli russelli*) venom another protein toxin drCT-II has been identified which also possessed antineoplastic activity. drCT-II treatment to HepG2 cell lines caused

increase in expression of p53 and moderate expression of p21/p27 suggested its apoptotic characteristic[22].

The presence of anticancer enzymes in snake venom was first mentioned during mid sixties[23,24]. Among them phospholipase is the lipolytic enzyme that hydrolyses the fatty acyl ester at the sn-2 position of membrane phospholipids that may mediate cytotoxicity. Phospholipase activity, found in cobra venom, was ascribed to be the enzyme with anticancer potential[25]. Phospholipase B in the venom of the Australian elapid snake was cytotoxic to cultured rhabdomyosarcoma cells[26]. Phospholipase A2 (PLA2), isolated from *Bothrops newweidii* venom, produced cytotoxic activity on B16 F10 melanoma cell[27]. Two toxic PLA2s have been purified from the Indian cobra (*Naja naja naja*) venom, which were neurotoxins but also showed cytotoxicity to Ehrlich ascites tumour cells[28]. Three other acidic phospholipases A2 were purified by successive chromatography of Indian cobra (*Naja naja naja*) venom. They were found to be cytotoxic to Ehrlich ascites tumour cells and devoid of lethality, haemolytic and anticoagulant activities[29]. Myotoxic phospholipases A2 isolated from *Bothrops brazili* snake venom displayed cytotoxic activity on human T-cell leukemia (JURKAT) lines[30]. Recently it was established that *Naja naja atra* phospholipase A2 mediated death of human neuroblastoma SK-N-SH cells was induced by upregulation of Fas and FasL, which was regulated by Ca^{2+} and ROS-evoked p38 MAPK activation[31].

L-amino acid oxidase (LAAO) occurs widely in snake venoms. LAAOs are the flavoprotein consisting of two identical subunits, each with a molecular mass of approximately 60 kDa. Tan and Fung[32] showed that LAAOs were multifunctional enzymes exhibiting edema-inducing, platelet aggregation inducing or inhibiting, apoptotic inducing as well as anti-bacterial, anti-coagulant and anti-HIV effects; these effects were mostly mediated by the H_2O_2 liberated in the oxidation process of the enzyme. LAAOs, isolated from *Ophiophagus hannah* venom[33] decreased [3H] thymidine uptake to murine melanoma, fibrosarcoma, colorectal cancer and Chinese hamster ovary cell lines that showed reduction in cellular proliferation[34]. ACTX-6, a LAAO, was isolated from *Agkistrodon acutus* snake venom. The homodimeric, 96 kDa, ACTX-6 showed accumulation of tumor cell at sub G1 phase of cell cycle[35]. It also induced apoptosis via Fas pathway in A549 cells. Activation of caspase-9 after ACTX-6 treatment demonstrated that mitochondrial pathway was also involved in ACTX-6-induced apoptosis. It was also found that the ROS scavenger catalase could inhibit ACTX-6-induced apoptosis. Thus C-Jun N-terminal kinase (JNK) pathway had been proved to be a necessity in ACTX-6-induced apoptosis and the oxidative stress generated by ACTX-6 was responsible for the activation of JNK[36]. This suggested the involvement of non-catalytic activity of the snake venom enzymes in the signaling pathway of cancer cell death.

The Arg-Gly-Asp- (RGD) containing disintegrins are non-enzymatic proteins that inhibit cell-cell interactions, cell-matrix interactions, and signal transduction. Disintegrins also possess the ability to inhibit several aspects of tumor cell behavior both *in vitro* and *in vivo*, including adhesion, migration, invasion, metastasis and angiogenesis[37,38]. Salmosin, a disintegrin isolated from Korean snake venom, effectively suppressed growth of metastatic tumour as well as solid tumour in mice[39]. Antimetastatic activity of salmosin, resulted from blocking the integrin-mediated adherence and αv, β3 integrin-mediated proliferation of the melanoma cells[40]. The anti angiogenic activity of salmosin has been demonstrated by protein neovascularization study in chick chorio-allantoic membranes and in matrigel implanted subcutaneously into mice[41]. The biological activity of condrostatin, a homodimeric disintegrin, isolated from copperhead snake venom, was found to be a potent inhibitor of *in vitro* β1 integrin-mediated M24 met cell adhesion and *in vivo* lung colonization[42].

Contortrostatin, a dimeric disintegrin, isolated from southern copperhead snake venom, prevented invasion of human breast cancer cells (MDA-MB-435) through an artificial matrigel basement membrane. It was not cytotoxic, rather it inhibited proliferation of the breast cancer cells by blocking αV β3, an important integrin mediating cell motility and tumor invasion, and perhaps other integrins and thus inhibited *in vivo* progression[43]. A novel disintegrin, Saxatilin, was purified from Korean snake (*Gloydius saxatilis*) venom[44]. Saxatilin, inhibited TNF-α induced proliferation of the ovarian cancer cells by suppressing activating protein-1(AP-1) dependent interleukin-8 expression and decreased cancer cell invasion by regulating MMP-9[45,46]. Another disintigrin, isolated from *Crotalus atrox,* Crotatroxin

2, inhibited human whole blood platelet aggregation, cancer cell migration, and experimental lung tumor colonization in BALB/c mice[47]. Colombistatin, isolated from *Bothrops colombiensis*, which inhibited ADP-induced platelet aggregation, human urinary (T24) and skin melanoma (SK-Mel-28) cancer cell adhesion to fibronectin, and cell migration[48]. Thus disintegrin of snake venom could progress as a therapeutic tool in the treatment of various cancers and thrombotic diseases in the near future.

Anticancer activity of scorpion venom / toxin(s)

Scorpion belongs to the class Arachnida under the order Scorpionida and phylum Arthropoda. Among all other members of the class Arachnida, scorpions are the most venomous representatives. Scorpion venom is a complex mixture of salts, small molecules, peptides, and proteins. Scorpion and its venom have been used as traditional and folk therapy in various pathophysiological conditions that has been mentioned in folk and traditional medicines of India, China, Africa and Cuba.

Wang and Ji[49] reported that *Buthus martensii* Karsch venom induced cell death of cultured malignant glioma U251-MG cells *in vitro* but did not affect human hepatocellular carcinoma cells and Chinese hamster ovary cells. Glioma cell death was determined as apoptosis by 4,6-diamidino-2-phenylindole (DAPI) staining and FACS analysis. *B. martensii* venom could significantly inhibit the tumor growth, which was assessed using U251-MG tumor xenografts on severe combined immunodeficiency mice. This venom induced apoptosis and inhibited growth of glioma, probably by inhibiting and modulating various ion channels in glioma cells[49].

Das Gupta *et. al*[50], established the cytotoxic activity of Indian black scorpion (*Heterometrus bengalensis*) venom on human leukemic U937 and K562 cells. Venom from this scorpion inhibited U937 and K562 cell growth characterized with membrane blebbing, chromatin condensation and DNA degradation in both the cells as evidenced by confocal, fluorescence, scanning electron microscopy. *H. bengalensis* venom induced DNA fragmentation as evidenced by comet formation. Flow-cytometric assay revealed a significant amount of early and late apoptotic cells due to scorpion venom treatment. The venom induced cell cycle arrest was observed with maximum cell accumulation at sub-G1 phase[50].

Scorpion venom contains proteins that specifically bind to the voltage sensitive Cl^- channels on cell membranes, known as Chlorotoxins. It was first isolated from the venom of the scorpion *Leiurus quinquestriatus*[51]. The peptide was 36 amino acids long and functionally it could bind to voltage gated Cl^- channels and brought about various changes in the cell physiology. It was toxic to insects but non-toxic to the mammalian system[52]. This toxin had a potency to bind to mouse epithelial cells and had got a unique ability to bind to glioma cells. Because of the later property, chlorotoxins are now used in treatment of glioma[53]. Chlorotoxin bind to xenograft tumors of mice with great specificity[54].

Lyons *et. al*[53], showed that chlorotoxin could bind to different primary brain tumors with more than 90% specificity. They also showed that chlorotoxin failed to bind to normal organs of the body suggesting that this could be a potentially harmless drug for normal and unaffected tissues in fighting cancer. Chlorotoxin bind to the matrix metalloproteinase-2(MMP-2) of the glioma cells prevented their activity[55]. MMP-2 was found to be expressed in several types of cancerous tissues including glioma, where it considered to be the main MMP. However, MMP-2 was absent in cells of normal brain tissue. Chlorotoxin has passed the preclinical safety tests and has achieved clearance from US FDA and at present in phase I/II clinical trials[56].

Sun *et. al*[57], had explored the future potential of Chlorotoxin as a non-invasive screening tool for early detection of skin, cervical, esophageal, colon and lung cancers. They suggested the usefulness of Chlorotoxin in identifying cancer positive lymph nodes that could mean a significant advancement for breast, prostate and testicular cancers. Chlorotoxins, conjugated with iron oxide nanoparticles through a polyethylene glycol linker, could successfully attach to both drug and targeting ligands. The target nanoparticle demonstrated preferential accumulation and increased cytotoxicity in tumor cells. Further, in *in vivo* models these nanoparticles were retained within tumors. It was suggested that this multifunctional nanoparticle system may find potential applications in cancer diagnosis and treatment[57].

Few scorpion enzymes, as serine proteinase and hyaluronidase, have been reported to have anti cancer activities. A serine proteinase-like protein named BMK-CBP, isolated from the venom of Chinese red scorpion (*Buthus martensii* Karsch) bind with the cancer cell line MCF-7 and the cell binding ability was dose-dependent[58].

Hyaluronidase, a virulent factor of beta-hemolytic streptococci and also present in the venoms of snake, bee, wasp, scorpion, etc, aids in the spread of venoms in the body. A homogenous hyaluronidase, named BmHYA1, was purified from the venom of Chinese red scorpion (*Buthus martensi*). The thirty N-terminal amino acids sequence of BmHYA1 was obtained by Edman degradation, which had no similarity to other venom hyaluronidases. Further, BmHYA1 could hydrolyze hyaluronic acid into relatively smaller oligosaccharides and modulated the expression of CD44, cell surface markers in the breast cancer cell line MDA-MB-231[59].

Cytotoxic activity of an anticancer polypeptide from *Buthus martensii* venom (APBMV) could augment natural killer cells activity, promote proliferation of lymphocytes, potentiate the response of delayed type hypersensitivity, antagonize the decrease of WBC in peripheral blood in the H22-bearing mice and thereby increased immune function in the H22-bearing mice[60]. ANti Tumor Peptide (ANTP) of molecular mass 6280 Da was purified by gel filtration chromatography and HPLC, from the venom of *Buthus martensii* Karsch that prevented proliferation of the mouse S-180 fibrosarcoma cells and murine EAC cells[61]. Charybdotoxin (CTX), a 37 amino acid neurotoxin from the venom of the scorpion *Leiurus quinquestriatus* hebraeus, induced blockage through Ca^{2+}-activated K^+ channels, caused a slight depolarization in human breast cancer cells and thereby arrested the cell in the early G1, late G1, and S phases and accumulated cells in the S phase[62].

Bengalin, a high molecular weight protein isolated from the Indian black scorpion (*Heterometrus bengalensis*) venom showed anticancer activity, on U937 and K562 cell. Bengalin elicited loss of mitochondrial membrane potential (MMP) which commenced cytochrome *c* release in cytosol, decreased heat shock protein (HSP) 70 and 90 expression. This showed that, bengalin might provide a putative molecular mechanism for their anticancer effect on human leukemic cells which might be mediated by mitochondrial death cascade[63].

Anticancer activity of toads and frogs venoms/ toxin(s)

Medical and pharmaceutical significance of purified compounds from toads and frogs skin and oocyte had been established[64,65]. The anticancer activity of crude toad skin extract was tried with Chan Su, a traditional Chinese medicine prepared from the dried white secretion of the auricular and skin glands of toad (*Bufo bufo gargarizans*). Chan Su-induced apoptosis in T24, human bladder carcinoma cell line. Chan Su treatment was coupled with a down-regulation of anti-apoptotic bcl-2 and bcl-X(S/L) expression and an up-regulation of pro-apoptotic bax expression. It induced the proteolytic activation of caspase-3 and caspase-9[66]. Huachansu, an injectable form of Chan Su, is a sterilized hot water extract of dried toad skin. Since 1991, Huachansu had been officially approved as a regimen for treatment of cancer in China. Cinobufocini injection, a preparation containing water soluble components of Chan Su, showed anticancer effects in clinical and experimental studies[67,68]. Recently Wang *et. al*[69] had established caspase 3 mediated and survivin regulated apoptotic activity of cinobufocini in lung cancer[69].

The skin extract (TSE) of common Indian toad (*Bufo melanostictus*, Schneider) possessed significant antineoplastic activity on EAC cells and human leukemic cell lines U937 and K562[70,71]. The cell growth inhibition due to TSE was established by G1 phase arrest in cell cycle of leukemic cells. Large number of early and late apoptotic cells were found in TSE treated leukemic cell colony as compared to the untreated cells[72].

Bufadienolides is one of the active constituents of Chan Su preparation. Bufadienolides and its more polar conjugate, the bufotoxins, are present in the skin of toad of the genus *Bufo*. The cytotoxic activity of toad bufadienolides was established on primary liver carcinoma cells PLC/PRF/5[73]. From Chan Su, five new bufadienolides, viz. 3β-formyloxyresibufogenin, 19-oxobufalin, 19-oxodesacetylcinobufagin, 6α-hydroxycinobufagin, and 1 β -hydroxybufalin, have been isolated together with previously known bufadienolides 6-20. All of them provided significant inhibitory activity against KB and HL-60 cancer cell lines[74]. 20S,21-epoxyresibufogenin, 20R,21-epoxy-resibufogenin, and 3-oxo-20S,21-epoxyresibufogenin, members of the rare epoxidized bufadienolides of Chan Su, significantly inhibited the leukemic MH-60 cell line[75]. A new bufadienolide named 16 β-acetoxy-bufarenogin was recently isolated from Chan Su, which showed *in vitro* cytotoxicity against HeLa cell line[76].

Bufalin, another bufadienolides from Chan Su, has anti-cancer property against leukemia as well as melanoma cells[77]. The initial stage of differentiation

and apoptosis induced by bufalin in K562 cell towards macrophage/monocyte was demonstrated by downregulating WT1 expression[78]. Bufalin arrested the growth of ML1 cells preferentially at G2 phase and U937 cells at the S and G2 phases of the cell cycle. Further, an increase in bufalin concentration noticeably inhibited DNA synthesis and topoisomerase II activity of cancer cells[79]. Bufalin produced cytotoxic and differentiation-inducing effects on human skin squamous cell carcinoma cells in *in vitro* experiment by decreasing its DNA synthesis[80]. Bufalin-induced apoptosis in cancer cells decreased time dependent gene expression of c-myc and bcl-2 genes with the relentless activation of MAP kinase acting through AP-1 activation[81,82]. Apoptosis induced by bufalin was associated with downregulation of protein expression, dephosphorylation, including cleavage of bcl-2 in HL-60 cells[83]. Bufalin also induced anti-tumor effect on the experimental hepatocellular carcinoma model of mice and human osteosarcoma cell lines by regulation of bax / bcl-2 and other regulatory protein expression in tumours[84,85]. At the genetic level, involvement of human gene Tiam1 in bufalin induced apoptosis through the Rac1, PAK, and JNK pathway had been established[86]. The inhibitory effects of standard anticancer agents, cisplatin and retinoic acid on cancer cell growth was potentiated after bufalin treatment and enhanced the induction of cell death[82].

Cinobufagin, isolated from *Bufo siccus,* showed *in vitro* inhibitory effect on five types of human cancer cells[87]. The cytotoxic activity of bufalin and cinobufagin on prostrate cancer cell lines was associated with constant increase in Ca^{2+}, leading to apoptosis[88]. Cinobufagin, one of the major constituents of huachansu, when treated along with gemcitabine-oxaliplatin showed helpful recovery of the patient with locally advanced or metastatic gallbladder carcinoma[89].

Magainin 2, a 23-residue alpha-helical defense antibiotic peptide molecule isolated from Xenopus, and its two synthetic analogues could rapidly and irreversibly lyse hematopoietic tumor and solid target cells through the concentrations that were relatively non-toxic to well-differentiated cells. The anti cancer activity of aurein peptides were established by the National Cancer Institute, USA[90]. Commonly, the cancer cell killing mechanism of such alpha helical defense peptides like aureins and magainins involved disruption of mitochondrial membrane integrity and/or that of the plasma membrane of the target tumour cells[91]. Maximins, isolated from skin secretions of *Bombina maxima*, were found to be cytotoxic to tumour cells, but at the same time they were toxic to mice[92]. A neuropeptide, Caerulein 1.1, has been isolated and identified from the secretions of the skin glands of the Stony Creek Frog *Litoria lesueuri*[93]. Citropin 1.1, and its other analogous synthetic peptide, A4K14-citropin 1.1, showed anticancer activity on 60 different human cell lines as tested by US National Cancer Institute[94].

A non-hemolytic defensin, Brevinin-2R, has been isolated from the skin of the frog *Rana ridibunda*[95]. It exhibited pronounced cytotoxicity towards malignant cells, including Jurkat (T-cell leukemia), B-cell lymphoma, colon carcinomas, fibrosarcoma, breast adenocarcinoma, lung carcinoma, as compared to primary cells including peripheral blood mononuclear cells, T cells and human lung fibroblasts. Brevinin-2R caused over-expression of pro-apoptotic molecules in these cancer cells. It also led to decrease in mitochondrial membrane potential and increase in reactive oxygen species without changing caspase activity. Autophagosomes have been detected upon Brevinin-2R treatment. These studies suggested that autophagy-like cell death due to Brevinin-2R treatment was activated by lysosomalmitochondrial death pathway[95].

One non-protein compound (BM-ANF1) was isolated from the skin extract of *Bufo melanostictus*. It possessed antiproliferative and cytotoxic properties. The cytotoxic property of BM-ANF1 was mediated through caspase-3 upregulation. BM-ANF1 also induced dose dependent expression of p53, the tumor suppressor protein which was corresponding to the expression of cyclin dependent kinase inhibitor $p21^{cip1}$ and $p27^{kip1}$ together with suppression of proliferating cell nuclear antigen, a regulator molecule of DNA synthesis. All these collectively supported the antiproliferative activity of BM-ANF1 by arresting cell cycle at G1[96]. Further, it was found that BM-ANF1 synergistically acting with Curcumin on colon cancer cells modulated its growth inhibitory properties[97]. A protein molecule (BMP1) has been isolated from the aqueous skin extract of toad (*Bufo melanostictus*, Schneider) skin which inhibited growth of EAC cells in mice and human leukemic cells[98].

Onconase from frog egg and oocytes specifically bound and internalized into the 9L glioma cells,

where it degraded RNA, inhibited protein synthesis and led to cytotoxicity[99]. In phase I study, the dose-limiting toxicity (DLT) of onconase, clinically known as Ranpirnase, was characterized by proteinuria (with or without azotemia), peripheral edema, and fatigue. The tumor-specific activity of ranpirnase was investigated in phase II trials in malignant mesothelioma, breast cancer, non-small cell lung cancer, and renal cell cancer. A Phase III randomized revision in malignant mesothelioma patients evaluated the combination of ranpirnase combined with a chemotherapeutic drug, doxorubicin to doxorubicin monotherapy. It was suggested that ranpirnase may be superior to doxorubicin in certain subsets of patients. Ranpirnase had demonstrated the ability to overcome P-glycoprotein mediated multidrug resistance in a human colorectal cancer cell line. It was also found that the antitumor activity of ranpirnase was p53 independent[100]. It was reported that *Rana pipiens* oocytes contained another ribonuclease, named amphinase, having similar activity to that of onconase[101]. Ardelt *et. al*[102], have postulated that, onconase and amphinase were highly cationic molecules with preferential toxicity towards cancer cells. The cancer cells having distinctly higher negative charge, compared to normal cells, increased binding efficiency of onconase and amphinase to the cell surface by electrostatic interactions.

Future trends of cancer research with venoms and toxins

Combination drug targeting, a key point in cancer signaling can set a new trend in chemotherapy. VRCTC-310, a combined product of two purified animal venom toxins, crotoxin and cardiotoxin showed antitumour property as was found by the National Cancer Institute and passed the phase I trial[103,104]. Another example of combination therapy using atroporin and kaotree isolated from the venoms of *Crotalus atrox* and *Naja naja kaouthia,* respectively, was documented by Lipps[105]. It was found that both of them individually killed a wide array of human (breast, colon, liver, ovary, etc.) and animal cancer cells but had no effect on normal mouse kidney, liver, spleen, and erythrocytes. Atroporin and kaotree complemented each other in combination to show elevated anti-cancer activity in *in vitro* and *in vivo* systems[105]. Combinations of toxins and herbal compounds too have promises. Curcumin, a major active ingredient of turmeric (*Curcuma longa*), has no discernable toxicity, inhibited the growth of transformed cells and colon carcinogenesis[106]. BM-ANF1 purified from skin extract from Indian common toad (*Bufo melanostictus* Schneider) inhibited growth of leukemic U937 and K562 cells and hepatoma (HepG2)[96]. Recently it was found that curcumin together with BM-ANF1 inhibited growth of colon cancer HCT-116 cells more than the inhibition caused by either agent alone. The combination of curcumin and BM-ANF-1 caused a marked inhibition of CDK2 and CDK4 and up regulation of CDKs inhibitors, p21 and p27. These events appeared to be p53 dependent and regulated *via* Akt-NF-κB signaling[97].

It can be suggested that the future prospects of cancer treatment lies somewhere in the combination therapy. The combination of product may be between (i) animal – animal (ii) animal – plant and (iii) animal – plant – synthetic compound. It should also be noted that combination may lead to the development of toxicities, which need to be evaluated along with its anticancer potential. Research in the next decade with venoms and toxins will definitely add information in the area of cancer biology and its management. It is obvious that these complex chemicals, derived from animal venom, could provide lead information to the biomedical scientist of cancer biology.

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