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BUFO SKIN-SECRETIONS ARE SOURCES OF PHARMACOLOGICALLY AND THERAPEUTICALLY SIGNIFICANT COMPOUNDS

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ABSTRACT

Amphibians have been occupying a wide range of habitats since they evolved around 363 million-years-ago. Along with legs and lungs, skin played an important role in survival of amphibians and made it possible for them to exploit diverse ecological conditions. Amphibian skin not only helps in avoiding desiccation but also helps in imposing defense against predators as well as pathogens. Amphibian skin possesses wide variety of chemical compounds, which have potential significance in pharmacology and therapeutics. Toads especially those belonging to genus Bufo, are outstanding source of useful granular-gland secretions. Compounds derived from toad skin-secretions can be used as analgesics, painkillers and as medicine against cardiac-problems, multi-drug resistant bacteria, HIV and Cancer.

KEYWORDS: Bufadienolides, pharmacology, Bufo skin-secretions, toxins.

INTRODUCTION

Amphibians started trolling the landmasses of earth about 363 million-years-ago, with Acanthostega and Ichthyostega probably being the earliest of known amphibians (Evans[20] et al 1998). Fossil records elucidate that ancestors of modern amphibians like Frogs, Toads, Caecilians and Salamanders probably evolved about 200 million years ago during the Triassic period. A very interesting observation here states that, fossils of these ancestral forms dating back to Triassic, tend to exhibit most phenotypic features of their living relatives (Wilson [64]et al 1974), implying that they haven't “evolved” much since then, owing to a structure, which seems to suite perfectly with their habitats. Amphibians evolved

from fishes and during this transition various evolutionary advances were produced in amphibians. While this transition produced many advanced systems like legs, it also produced specialized alveolar or tubular glands in amphibian skins, which is sometimes considered as amphibian's chief evolutionary advance over that of fish-integuments (Noble[45] 1931). These specialized glands had many functions, including secretion of substances having poisonous properties for repelling or killing predators as well as microbial pathogens. This evolutionary advance made amphibians one of many other organisms in the animal kingdom, which indulged in chemical wars (Hoiberg[29] et al 2002; Daly[15] et al 2005) in order to survive in the battle for survival (See Table.1.). In the following sections various properties, effects and perspectives regarding toad skin-secretions have been discussed.

Host defense peptides (HDPs) are endogenous antibiotics secreted by the holocrine glands, play multifunctional role in the innate immunity of vertebrates and other organisms. These effector molecules are rapidly produced ribosomally in response to infections and defend the animal from invading pathogenic microorganisms. Majority of them are cationic in nature, vary in chain length, possess amphipathic structure and exhibit broad-spectrum antibacterial, antiviral, antifungal and anti-inflammatory activities and. Their wide spread distribution in the animal and plant kingdom suggest that they have served a fundamental role in the evolution of complex multi-cellular organisms. Since majority of these peptides target the microbial membrane, the chance to develop resistance against them is negligible compared to conventional antibiotics. Therefore these molecules can serve as a potential candidate to counter the menace of emerging multidrug resistant strains of pathogenic microorganisms.

Extensive analysis of the frog skin and its secretion identified several bioactive peptides that defend the animals from microbial attack. The genus ***Rana*** is the most diverse and widely distributed group of anuran amphibians, with more than 250 reported species around world. They posses many HDPs with broad spectrum antimicrobial activities like gaegurins and rugosins of ***Rana rugosa,*** brevinins of ***Rana brevipodaporsa***, ***Rana esculenta*** and ***Rana sphenocephala*** , esculentins of ***R.esculenta*** , ranalexin and ranatuerins of ***Rana catesbeiana***, and temporins of ***Rana temporaria***. Unfortunately this study was limited only to frog species of the temperate region and only a little is known about the nature of molecules present in tropical frogs. The Satakosia Tiger Reserve of central Odisha, India is one of the major biodiversity hotspot of the world with high degree of endemism. A detailed exploration of the defensive frog peptides of this region will help to understand their amino acid assembly,

secondary structure and valuable information about their mode of action which may help to understand the minimum structural requirement of a peptide to show antibacterial activity. These molecules may also serve as a template to develop novel anti-infectives with predicted bioactivities.

HDPs isolated from Ranid frogs have very high sequential similarity and are grouped under Brevinin family. These peptides contain a unique primary structure in which N-terminal is a linear segment and the C-terminal is a seven membered cyclic region formed by a disulfide bond between ***i*** and ***i*** + 6th cysteine residues. The present study describes the isolation, characterization and antibacterial activity of five novel skin secreted HDPs from the Indian ranid frog ***Clinotarsus curtipes***. Structural analysis showed that these peptides possess very high sequence homology with known brevinin1 family peptides isolated from Ranidae frogs of Eurasian and North American region. A detailed analysis of their primary and secondary structure, biological activity and mechanism of action will be useful to design and develop novel peptides based anti infectives of known activity.

Figure 1

Bufo melanostictus

MATERIALS AND METHODS

Collection of frog skin secretions

Skin secretions were collected from twenty adult species of *B. melanostitus* (20–35 g; sex unknown) of the Satakosia Tiger Reserve of central Odisha , India as previously described. In brief, a mild electric stimulation was given to the dorsal surface of the Toad. The skin secretion was collected by washing the toad skin with small quantities of sterilized, deionized water acidified with trifluoroacetic acid. The toad was released in a healthy state back to field

where it was collected. Skin secretions were pooled together, frozen in liquid nitrogen, brought to the laboratory and lyophilized.

Table 1: Animals indulging in Chemical Warfare Race

Animals	Principal poisonous substances
Cnidarians	
Portuguese Man of –war(Physalia)	Tetramine,5-hydroxy tryptomine
Sea wasp(*Chironex* f*lecker*i)	Cardiotoxin
Arthopods	
Honey Bee (*Apis* sp)	Melittin, Hyaluronidase
Millipedes (*Apheloria* sp)	Hydogen cyanide, Benzaldehyde
Scorpion (*Centruroide*s sp)	Cardiotoxin, Lecithinase
Blister Beetles (*Cantharis vesicatore*a)	Cantharidin
Ants	Pumilotoxins Izidines Lehmizidines
Molluscus	
Turban shell (*Turbo argyostoma*)	Ciguatoxin
Octopus (*Octopus maculosus*)	Cephalotoxin
Pisces	
Moray eel (*Gymnothorax javanicus*)	Ciguatoxin
Castor oil fish (*Ruvettus pretigus*)	Oleic acid
Puffer fish (*Arothron hispidus*)	Tetradotoxin
Sting ray (*Dasyatis sp)*	Sting ray venom
Amphibians	
Salamander (*Salamandra maculosa*)	Salamandarine, Salamandenone,Samanine
Newt (Tancha torosa)	Tanchatoxin
Frogs	Batrachotoxins, Histrionicotoxins
Toads	Bufadienolides

Specialized glands of Toad-skin

Amphibians like toads possess two types of alveolar glands in inner-layer of stratified epidermis of their skin: mucous glands and granular glands (Noble[45] 1931; Hickman[25] et al 1995). Mucous glands are scattered all over the body and secrete a transparent mucus secretion acting as lubricant in water and also helping in keeping skin moist on land. This mucus may contain many glycoproteins like mucins, mucinigen and carbohydrate-residues like galactose, fucose and sialic acid (Williams[63] et al 2000). Granular glands on the other hand are "serous" type (Hickman[25] et al 1995) having centrally placed nucleus and secreting acrid poisons or toxins, which help in providing protection from predators like birds, mammals, snakes, crocodiles who try to eat them (Storer[58] 1925; Awasthi[4] 2006). Granular glands usually require considerable stimulation to produce their milky poisonous secretions (Noble[45] 1931) and may be found arranged in form of clustered pads e.g. Parotid glands of common toads. Secretions of mucous glands stain with basic dyes and lack granular

appearance while that of granular glands has granular appearance and stain with plasma dyes. Toad granular gland secretions generally induce very serious inflammations of eyes or digestive tract, unpleasant experience and vomiting sensations in toad predators (Pough[51] et al 1999; Biedermann[7] 1930). Cane toad (Bufo marinus) is probably the most venomous of all other toads. Instances of snakes or domestic animals found dead with cane toad in their mouths or guts have been reported. Cane toads have also been found to influence the population of monitor lizards and various other frogs or toads (Hinkley[62] 1962). Such is the impact of these venomous creatures that some animals have evolved strategies to avoid these toads e.g. two snake species have been reported to develop smaller heads so that they can no longer eat venomous cane toad in Australia (Awasthi[4] 2006; Aldous[2] 2004). Even human beings have been reported to die after consumption of mixture of toad skin-secretion components (Barry[5] et al 1996). Toad skin toxin's potency is so profound that hedgehogs (Insectivora, Erinaceidae) use them for enhancing their own mechanical anti-predatory adaptations (Brodie Jr[10]. 1977). Hedgehogs take these secretions into their mouths and lick it on their spines. Hedgehogs use them since presence of such substances in fresh or dried form on their spines probably increases the pain or potential of infection in its predators. But toads don't enjoy the merit of being the most poisonous amphibians; rather this title is a proud property of poison-dart frogs (Phyllobates terribilis). These frogs secrete a neurotoxin called batrachotoxin, which is so fatal that South-American Indians use it to cover the tips of their arrows for hunting (Daly[15] et al 1992). However in regions like Europe where such frogs aren't found toad may be the most poisonous, so much so that in medieval Europe toad's skin-gland extracts were employed in witchcraft (Hofrichter[27] 2000).

Toad-toxin's Chemistry

Toad secretions of both mucous and granular glands can sometimes be poisonous (Phisalix[50] 1918) however granular glands produce more toxic secretions than mucous glands. Granular gland secretions in toads contain chemicals that can be broadly classified into four categories: (1) Biogenic amines, (2) Bufadienolides, (3) alkaloids and steroids and (4) peptides and proteins (Clarke[12] 1997). Chemically, other than biogenic amines and peptides, granular gland secretions of toads may contain nearly 86 types of Bufadienolides (Steyn[57] and Heerden 1998) along with other components like Bufotoxin, Bufagin and Bufotenine (Morris[42] 1992; Hoiberg[29] et al 2002; Hirai[27] et al 1992). Bufalin, Bufogenin, Bufotalin, Cinobufagin, Marinobufagin, Resibufagin are some of the most important bufadienolides. Major bufadienolides isolated from skin secretions of Bufo species are listed in Table

2. Biogenic amines present in toad-secretions include serotonin (5-hydroxytryplanime), histamine, bradykinin etc. (Basir[6] et al 2000).

Table:-2

Arenobufagin	Cinobufotalitoxin
Arenobufagin hemisuberate	Desacetylcinobufotalin
Arenobufotoxin	Gamabufotalin
Argentinogenin	Gamabufotalitoxin
Bufalin	Hellebrigenin
Bufalin hemisuberate	Hellebritoxin
Bufalitoxin	Marinobufagin
Bufotalin	Marinoic acid
Bufotalinin	Marinosin
Bufotalone	Resibufagin
Cinobufagin	Resibufaginol
Cinobufagin hemisuberate	Resibufagenin
Cinobufagino	Resibufotoxin
Cinobufotoxin	Telocinobufagin
Cinobufotalin	Vulgarobufotoxin
References :Steyn and Heerden 1998	

Bufadienolide is regarded as derivative of steroids with two double-bonds present in bufanolide side-chain such that it's known as bufotoxin when in combined-state with nitrogenous compounds like arginine and bufogenin when in free-state (Hoiberg[29] et al 2002). Bufadienolides occur both in nonconjugated as well as in conjugated form (Steyn[57] and Heerden 1998). Conjugation may be present at carbon number 3 to give rise to sulfates, dicarboxylic esters and amino-acid dicarboxylic acid esters. Various substitutions in the side-chain or side groups of bufadienolides can give rise to many types of derivatives and such substitutions may vary from specie-to-specie. Bufadienolides are also responsible for imparting a milky appearance to the skin secretions of toad. Toad skin glands actually secrete a compound called bufonin, which is a weaker poison but as soon as it comes in contact with air it gets oxidized a to form a bufadienolide called Bufotalin [C34H46O10] which imparts a milky appearance to the toad secretions (Noble[45] 1931). Bufadienolides or compounds with similar structures are not only found in toads but also in many plant species, fireflies (Photinus sp.), Snakes (Rhabdophis sp.) and mammals (Steyn[57] and Heerden 1998). Apart from bufadienolides, other components of toad skin secretions like bufotenine and bufogen have also been well characterized. Bufotenine [$C_{12}H_{16}N_2O$] a crystalline solid, which is

insoluble in water but soluble in alcohol (Morris[42] 1992), while Bufogen [$C_{18}H_{24}O_4$] as well as bufotalin resemble the drug digitalis in their action mechanisms (Abel[1] and Macht 1912).

Alkaloids, steroids and bufadienolides are major constituents of toad skin secretions followed by proteins. Toad secretions contain many proteins which exhibit antimicrobial properties, enzymatic properties and help in repair of bruises or abrasions. Proteins that exhibit antimicrobial properties have been discussed in details in later sections. Other than normal proteins that are found in skin secretions of all toads there exists a distinct family of proteins that are secreted only by the toad genus called Bufo. These proteins, which also possess germicidal activity, have been identified as, Bufotenine and were first found in toad (Bufo melanostictus). These proteins have about 28 amino acids and possess an amphipathic alpha-helical structure (Mor'[41] et al 1994). BLP-1, BLP-2 and BLP-3 are three bombinin-like peptides isolated from skin of B. orientalis (Gibson[21] et al 1991). All three peptides shared considerable homology. BLP-1 is the most abundant member of the trio. All these BLPs have also been found to consist of high helical content (63-69% alpha-helix) in their chemical structure.

Toad-toxins: Physiological Effects

Toxins secreted by toad skins exhibit wide spectrum of effects and their composition as well as effects differ from specie-to-specie (Wright[65] 1914; Pough[51] et al 1999). These toxins are primarily meant to act like venoms for protecting toad from predators however they also assist in protection from microbe-laden hostile habitat (Zaslof f[68] 2002). These toxins defend naked skin against microorganisms as well as assist in wound repair (Simmaco[56] et al1998). Some amphibians warn about their poisonous nature by virtue of bright skin-colors but, such colors might not always give an idea regarding virulence of secretions in case of toads, e.g. Large Leptodactylus pentadactylushas bright thighs but lacks highly poisonous secretions of drab-colored Bufo marinus (Brazi l[9] and Vellard 1926).

Toad-toxins act by inducing various physiological effects on higher as well as lower vertebrates. Granular gland secretions when entered into stomachs of higher-vertebrates cause nausea, weakening of respiration and muscular paralysis, while in contact with eyes they produce serious inflammations (Abel[1] and Macht 1912). Toad secretions may also boast of adrenalin, which is a result of chemical change within mature secretion (Shipley[55] and Wislocki 1915). Clinical aspects of toad-toxins have been studied particularly in Bufo marinus. Secretions of B. marinus are cardioactive, due to activity of bufogen and bufotalin

exhibiting clinical symptoms like dermatitis, hypotension and severe arrythmia (Radford[52] and Gillies 1986). Toad-secretions have also been found to show second-degree Wenckebach atrioventricular block and T-wave change (Lin[36] and Lin 1989). Other than heart associated activity, some toad secretions have also been found to exert effects on neurological activities. Skin extracts of Bufo melanostictus (common Indian toad) have been shown to contain sleep inducing factors (SIF) which induce sleep probably by alteration of brain biogenic amine levels, monoamine oxidase (MAO) and tryptophan hydroxylase (TH) activity (Dasa[16] et al 2000).

Toad skin secretions show cardioactive effect since they interact with the enzyme Na^+/K^+ ATPase. Normally in organisms like humans, inhibitors of Na^+/K^+ ATPase are involved in water and electrolyte homeostasis as well as in the genesis of vasoconstriction in volume-dependent forms of hypertension (Alexei[3] et al 1998). While such inhibitors are endogenous in other cases, amphibians especially those belonging to family Bufonidae have been shown to secrete compounds that inhibit Na^+/K^+ ATPase activity as well as antagonize the binding of ouabain to the enzyme from their skin (Flier[21] et al 1980). These compounds play important part in defense against predators. Bufalin, an active component of toad-secretions is a potent Na^+/K^+ ATPase inhibitor, which binds to cell membrane with higher affinity then ouabain (Jing[34], Watabe et al. 1994). Bufotalin inhibits myocardial Na^+/K^+ATPase activity, thereby increasing myocardial contractile force without affecting the heart-rate (Hirai[27] et al 1992). Marinobufagenin (3, 5-dihydroxy-14, 15-epoxy bufadienolide), marinoic acid and resibufogenin show Na^+/K^+ATPase inhibiting properties (Bagrov[4] et al 1995; Matsukawa[40] et al 1996; Pamnani et al 1994). While marinobufagenin is also a potent vasoconstrictor, resibufogenin (3-hydroxy-14, 15-epoxy-20, 22-dienolide) shows electrophysiological properties similar to acetylstrophanthidin (AS), which suggests similarity to family of digitalis-like drugs (Xie[66] et al 1994).

Bufotenine on the other hand, has been found to be an Indole-hallucinogen, which can block action of serotonin (Hoiberg[29] et al 2002). It can also constrict blood-vessels. Bufotenine-like compounds have also been isolated fromAmantia muscaria (mushroom) and Piptadenia peregrina (plant). But, bufotenine isn't the most potent hallucinogen found in toad skin secretions; this title is possessed by 5-methoxy-N, N-dimethyltryptamine (5-MeO-DMT), an active component of skin secretions of Bufo alvarius (Weil[62] and Davis 1994). While these skin secretions of B. alvarius are fatal when consumed orally yet they may be safely smoked

to experience their psychoactive power. Due to this property ancient peoples of Mesoameria probably used these toads as ritual intoxicants.

Toad-toxins: Antimicrobial Effects

Vertebrates and other organisms boast of distinct groups of broad-spectrum antimicrobial substances in addition to immune-systematic responses and toads are no exception to this evolutionary creation (Boman[8] 1995). The only difference though can be that, while other organisms and mammals like humans secrete such antimicrobial chemicals inside their body's coelomic-systems in form of ?-defensins or azurocidin (Nathan[43] 1987), toads secrete them both inside their body as well as outside their body into the environment through skin-pores. Toad-toxins secreted from such skin pores assist toads to survive in habitats full of pathogenic microorganisms which may cause diseases like Red Leg Syndrome, Mycobacteriosis, Salmonellosis, Chromomycosis and Saprolegniasis (Fox[22] et al 1984).

Research on antimicrobial effects of toad skin secretions started mainly in 1980s. Since then only certain bufadienolides and proteins have been found to show antimicrobial properties. Search for more such compounds still continues. Bufadienolides have only been recently found to exhibit antimicrobial properties. Bufadienolides like telocinobufagin and marinobufagin isolated from skin secretions of the Brazilian toad Bufo rubescens have been shown to exhibit anti-microbial activity. These compounds were shown to have inhibitory action over Staphylococcus aureus and Escherichia coli (Cunha[13]-Filho et al 2005). As compared to bufadienolides, toad skin secretions seem to possess more diverse type of proteins, which act as antibiotics. Antimicrobial peptides released by skins of toads are capable of causing lysis of many pathogenic bacteria, viruses, gram-positive and gram-negative bacteria, protozoa, yeasts, and fungi. Such antimicrobial peptides have characteristic chemical properties such as relatively small size (20-46 amino acid residues), basic nature (lysine- or arginine-rich), and amphipathic properties (Nicolas[44] and Mor 1995). Proteins called Bombinans and BLPs bombinin-like proteins) were probably first antibiotic proteins to be isolated from toad skin secretions. They were isolated from skin of Bombina orientalis. Bombinans secreted by Bombina orientalis have significant antimicrobial and antifungal. An unnamed toxin of molecular weight 6,700 isolated from skin secretions ofBombina sp. has been shown to exert antibacterial effects against both gram-positive and gram-negative bacteria (Mastromei[38] et al 1991). On the other hand, BLPs were found to be more potent then magainin 2 (antimicrobial peptide isolated from Xenopus laevis) in their effectiveness to

kill bacterial cells (Gibson[24] et al 1991). In members of genus Bufo, antimicrobial activity has been found to be mediated by lectin like proteins. A protein called β-galactoside binding lectin obtained from skin secretions of Bufo arenarum has been found to exhibit bacteriostatic activity (Riera[53] et al 2003). This protein, which is also found in ovary, oocytes and embryos of B. arenarum (Elola[18] et al 1998) has been shown to exhibit bacteriostatic activity against Escherichia coli K12 4100, wild strains ofEscherichia coli, Proteus morganii, and Enterococcus faecalis. rty, attributed to its amphipathic ?-helix (Mor[41] et al 1994). An unnamed toxin of molecular weight 6,700 isolated from skin secretions ofBombina sp. has been shown to exert antibacterial effects against both gram-positive and gram-negative bacteria (Mastromei[38] et al 1991). On the other hand, BLPs were found to be more potent then magainin 2 (antimicrobial peptide isolated from Xenopus laevis) in their effectiveness to kill bacterial cells (Gibson[24] et al 1991). In members of genus Bufo, antimicrobial activity has been found to be mediated by lectin like proteins. A protein called β-galactoside binding lectin obtained from skin secretions of Bufo arenarum has been found to exhibit bacteriostatic activity (Riera[53] et al 2003). This protein, which is also found in ovary, oocytes and embryos of B. arenarum (Elola[18] et al 1998) has been shown to exhibit bacteriostatic activity against Escherichia coli K12 4100, wild strains of Escherichia coli, Proteus morganii, and Enterococcus faecalis.

While anti-microbial activity of toad secretions is evident from above discussion, these secretions have been recently found to contain anti-viral activity. A 63 kDa heme-binding protein called BAS-AH isolated from Bufo andrewsi has been shown to exhibit anti-HIV activity (Zhao[69] et al 2005). At concentrations that have little effect on cell viability, it has been shown to inhibit recombinant HIV-1 reverse transcriptase activity. Research on anti-fungal and anti-parasitic effects of toad skin toxins is still under way.

Exhibition of microbicidal effect of the protein could be a result of its ability to form channels or pores within the microbial membrane causing permeation and lysis of the cell. While mechanisms by which bufadienolides exert their bactericidal effect are still debatable the effect of antimicrobial peptides present in these secretions seems to be understood. Since these peptides are generally cationic, they have been proposed to mostly act by disrupting and permeabilizing the target cell membrane (Shai[54] 2002) which thereby prevents the target organism from developing resistance to this defense mechanism, implying a potential therapeutic use of these peptides (Jacob[32] and Zasloff 1994). Gene families coding for such

antimicrobial peptides show gene diversification resembling gene families coding for immunoglobulins (Hughes[31] 1997) or venom-derived toxins (Ohno[47] et al 1998). Antimicrobial peptides isolated from these organism's skins differ considerably in their sequences from one toad to another or even amongst amphibians (Simmaco[56] et al 1998). Such divergence within species suggests that there may be existing about 100,000 different proteins secreted through dermal-glands of nearly 5000 different anurans (Duellman[17] and Trueb 1994).

Bufo-toxins: Pharmacological and Therapeutic Significance

Pharmacological potential of toad secretions was known to certain ancient medical-practitioners since some Chinese and Indian traditional medicinal procedures have been known to make use of Toad secretions. “Kyushin” is traditional Chinese medicine making use of toad-secretions and also exhibiting digoxin-like effects (Fushimi[23] et al1990). This cardiotonic effect is due to toad-toxin component “Chan'su”, a Chinese term for mixture of various toad-toxins specially, bufalin and cinobufagin. Problem with such medicines are that the quality and quantity of compounds shows great variability, which could be hazardous when in overdose (Hong[30] et al 1992). Ayurveda, the traditional-medicinal practice of India has prescribed rubbing of live toad on swollen areas of animal (cattle) throat for healing and removal of throat obstruction arising due to swelling (Tiwari[59] and Pande 2004). This is related to Agnikarma practice of Ayurveda. Toad skin secretions are source of diverse kind of pharmacologically and therapeutically significant compounds. These secretions contain components that can be used for production of painkillers, antimicrobial drugs, anti-viral drugs and even anti-cancerous drugs. These compoundscould also prove to be useful in treatment of cardiovascular ailments and neurological problems.

Toad skin secretions have been shown to contain components, which exhibit analgesic and pain killing effects. A component of toad-toxin called bufalin, which is a Na^+/K^+ ATPase inhibitor has in controlled studies of hepatic-cancerous pain, shown analgesic effects (Wang[60] et al 1994). Experimental results on bufalin action have shown that, it increases hepatic-blood circulation, which decreases amount of stagnating blood thereby decreasing pain (Wang[60] et al 1994). At present aspirin and morphine are drugs that are frequently used as analgesics. But aspirin has certain side effects, which include bleeding in stomach, Reye's disease, urticaria and asthmatic attacks (Brown[11] 2001). Morphine also has many ill effects. In this scenario, bufalin can be of potential usage in pain-relief, anxiety diminishing and sleep

induction. It may also substitute role of aspirin in treatment of rheumatic fever and arthritis. Other than bufalin, sleep-inducing factors (SIF) isolated from Bufo melanostictus can also be used for sleep induction (Dasa[16] et al 2000).

As discussed in previous sections, certain components of toad skin secretions which resemble digitalin and digitoxin in their structure show effects on cardiovascular system as well. These components can be used in place of digitalis-like drugs to increase strength of heartbeat and are thus useful in cases where cardiac rhythm is abnormal especially in atrial-fibrillation (Brown[11] 2001). Such compounds can also be used in treatment of hypertension and congestive heart failure (Oldfield[48] 1995). The most interesting aspect in therapeutic usage of components present in toad skin secretions other than their antimicrobial effect is their anti-cancerous activity. While multi-drug resistant bacteria are potential candidates against which these toad toxins can be used, another big problem, which would probably be solved by these toxins, could be cancer.

A number of bufadienolides have been recently shown to exhibit anti-cancerous properties in vitro. Components like Bufalin have been shown to exert anti-cancerous properties on leukemia cells lines. Bufalin was found to induce apoptosis in human-leukemia cells by altering expression of apoptotic genes c-myc and bcl-2 (Jing, Ohizumi[33] et al 1994; Masuda[39] et al 1995; Watabe[61] et al 1996). This effect of bufalin on cell cycle of leukemia cell resembles effect of topoisomerase inhibitors (Jing, Watabe[34] et al 1994). The concentration at which bufalin induced apoptosis in HL-60 cells was 10^{-8} M, which is comparable to drug camptothecin but lower than other anti-tumor drugs like cisplatin, VP16 and all-trans retinoic acid (Jing, Ohizumi[33] et al 1994). Cinobufagin, another bufadienolide has been shown to work in treatment of infection and granulocytopenia during combined chemotherapy (Hirai et al1992). It reduced the risk of infection and degree as well as duration of granulocytopenia associated with malignant blood diseases (Yue[67] 1992). Other than bufalin and cinobufagin certain other toad skin-secretion components like 3-formyloxyresibufogenin, 19-oxobufalin, 19-oxodesacetylcinobufagin, 6-hydroxycinobufagin and 1-hydroxybufalin have been recently found to exert inhibitory effects on KB, HL-60 and MH-60 cancer cell lines (Nogawa[46] et al 2001). Similarly, Bufogenin derivatives from skin secretions of Bufo bufo gargarizans as well as several semi synthetic derivatives of 20,21-epoxy-resibufogenin have been found to exhibit interleukin-6 (IL-6) antagonistic activity due to their growth-inhibitory activities on IL-6-dependent MH-60 cells (Enomoto[19] et al 2004).

However, anti-cancerous properties of toad skin-secretions can be debatable especially those on skin cancers because while they seem to inhibit cancer proliferation in vitro, toad themselves have been reported to be affected byskin cancers (Manskikh[37] 2003). This raises doubts on anti-cancerous activity of these secretions in vivo.

CONCLUSION

Toad skin-secretions are potent source of drugs. It's probably the only such source in nature from where we can get nearly six types of drugs possessing analgesic, painkiller, antibiotic, anti-viral and anti-cancerous properties as well as possessing potential of treating cardiovascular diseases. We know that cancer, RNA virus causing pandemics/epidemics and multi-drug resistant bacteria are prime problems of 21 st century and toad secretions have been shown to inhibit growth of cancer, HIV as well as super-bugs like Staphylococcus aureus. Since potency of these toxins differs from specie to specie among Anurans, we may have about 1, 00,000 such compounds still waiting to be discovered. But, we may loose more than 50% of them in next 20 years itself, if we don't stop destroying the habitats of these Anurans. In recent decades, amphibian populations around the world have experienced dramatic declines, with some species showing high rates of deformities and others simply disappearing. In fact some scientists fear that forces that threaten amphibian population may be fueling an increase in prevalence of infectious diseases like SARS and Lyme disease (Kiesecker[35] et al 2004). We must save fledging population of amphibians as well as other animals or else, we are not only loosing the integrity of our ecosystem but also potential sources of drugs. This is significant since many types of exotic viruses and super bugs are still unknown to humankind.

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