PLANT CELL CULTURE AS AN EMERGING ALTERNATE TECHNOLOGY FOR IN VITRO PRODUCTION OF ANTI SNAKE VENOM COMPOUNDS: A REVIEW

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ABSTRACT

Snake bite causes a severe health problems finally leading to death mainly in farm lands, and agricultural fields. The incidence of death rates caused due to snake bites reached an unestimated rate worldwide. Till date, the treatment of snake bite is dependent on poly valent anti-snake venom. In view of the current situation anti-snake venom is in tremendous demand due to its lack of availability. Medicinal plants are present all over the world that can be used as an antidote for snake bite treatment. This article includes prevalence of snake bite and bioactive compounds from medicinal plants that are efficient as anti-venom through molecular docking studies. So therefore, plant remedies might be beneficiary to human and discover a new substitute as antidote. Large scale production of these compounds through plant tissue culture can influence the rise of new drugs for therapeutic use which is effective, available, and definitely with no side effects.

KEYWORDS: Snake Bite, Venom, Antidote, Medicinal Plants

ABBREVIATIONS

PLA₂ Phospholipase A₂

AIDS Acquired Immuno Deficiency Syndrome

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INTRODUCTION

Snake bite is a severe health problem among public around the world mainly in Asia, Africa, Latin America and other parts of Oceania [1]. As a result, till now the incidence caused by snake bite is estimated around 2,00,000 people are affected and 35,000-50,000 people die every year in India alone [2]. Snake venom comprises of more than 20 toxic enzymes in snakes present all over the world and out of these 12 are found in all snake venoms [3]. It is a universal alarm in diverse locations of the globe particularly in tropical and subtropical regions [4]. The 12 enzymes are Phosphodiesterase, Phospholipase A₂ (PLA₂), L- Amino acid oxidase, Peptidase, NAD-Nucleosidase, Arylamidase, Ribonuclease, Deoxyribonuclease, Adenosine triphosphatase, 5' Nucleotidase, Phosphomonoesterase and Hyaluronidase [5]. The main objective of the study is to discuss about snake bite and production of therapeutic

compounds through tissue culture technology for treatment.

EPIDEMIC STATISTICS ON SNAKE BITE

The global burden of snake bite is often underestimated. Concerning about the number of snake bites incidence and the related mortality in India, WHO has reported several amounts of deaths in rural communities. In Jammu, the deaths caused by envenomation of snake bite by *Echiscarinatus* reached about 95% and it's found in India and Pakistan [6]. Gaitonde and Bhattacharya during 1980 reported that 70 bites per 1 lakh and deaths more than 2.4 per 1 lakh per year in Maharashtra [7]. It's estimated that over 10,00,000 snake bites where in India alone leading to 45,000-50,000 deaths annually due to toxicity of venom [8]. A study conducted on Paschim Medinipur district of West Bengal, overall 17 deaths were reported based on hospital data from 10 blocks between 2012 and 2016 [9]. In Egypt, a total of 87 snake bites caused by venomous and non-venomous species were recorded from January to December 2015, in NECTR (National Centre for Clinical and Environmental Toxicology) [10]. In America, an average incidence of snake bite is about 57,500 annually (6.34 per 1,00,000 population), resulting in almost 370 deaths (0.037 per 1,00,000 population) with a case fatality rate below 0.6% [11]. Recently, according to WHO (World Health Organization), around 81,410-1,37,880 people die and about three times as many amputations and other permanent disabilities are caused due to snake bite every year [12]. Incidence of snake bites showed most number of deaths in Mahbubnagar district and least amount of deaths in Srikakulam district of Andhra Pradesh [13]. In Kerala, 34% of patients develop total envenoming due to snake bites [14]. Among different types of snakes, saw-scaled vipers (*E. sochureki* and *E. carinatus*) are one of the vital species present in open and parched environments [15].

In India, one death caused by snake bite is equal to two deaths caused by AIDS [16] when compared to that of severe to highly infectious diseases [17]. According to the statistical data of snake bite in agricultural field is about half the victims and around 36.8% in living places in Tamil Nadu [18].

TYPES OF VENOMOUS SNAKES

The neurotoxic venomous snakes include coral, sea, cobra, mambas and kraits, while the haemotoxic venomous snakes are copperhead, rattle snakes and cotton mouths [19]. Venomous snakes are broadly classified into different families like Viperidae, Elapidae, Hydrophidae and Atractaphidae [20]. The most repeatedly found snakes in India are four different types; they are Indian cobra (*Najanaja*), Krait (*Bangarus caeruleus*), Russell's viper (*Daboia russelli*) and Saw-scaled viper (*Echiscarinatus*) which are venomous [21]. Apart from these, other poisonous snake species in India include, Asian cobra (*Naja xianana*), monocellate cobra (*N. kaouthia*), greater black krait (*Bungarus niger*), Wall's and Sind krait's (*B. walli* and *B. sindanus*), and West and hump nosed pit viper (*Hypnale hypnale*) [22].

Snake Venom

Venom in snake causes damage of local tissues, hemorrhage and myonecrosis [23]. Snake venom composes of toxic substances broadly classified into neurotoxins (affects central nervous system) and haemotoxins (affects circulatory system) in humans [24]. In addition, treatment of snake bite is one of the universal problem taken as serious matter by scientists and researchers for its cure [25]. Venom in snakes is a composite of biologically active compounds like proteins (enzymatic and non-enzymatic), peptides, lipids, nucleotides and carbohydrates. Some of the proteins are medicinally important but most of them are toxins [26]. It consists of inorganic cations like zinc, calcium, sodium, magnesium,

potassium, nickel, iron, etc for the activation of different enzymes in the body. For ex: zinc and calcium is responsible for activation of anticholinesterase and phospholipase enzymes [27].

Drawbacks on Poly Valent Anti-Venom

Hence, insufficient supply of anti-venom for deaths caused by snake venom is most likely to remain as one of the most quantifiable setback [28]. Plasma fractions obtained from immunized animals (usually horses) are used as anti-venoms [29]. Anti-venom from medicinal plants has become one of the leading purposes for the treatment of snake bite. It is a venom neutralizing Ab (immunoglobulins). The lone treatment for snake bite is immunotherapy [15]. Nowadays, conventional medicine is developed using medicinal plants by means of phytochemical compounds present in the plant for snake bite treatment. The treatment is controlled only by the use of anti-snake venom, where Alberte Calmette developed the first anti snake venom against Indian cobra [27]. The treatment for venom can be prepared using herbal plants. And mostly now in India, due to the lack of availability of anti-serum people turn to traditional medicine [30]. Major problem encountered with anti-venom is low efficacy in neutralization of venom components which causes local haemorrhage, necrosis and tissue damage in victims [31].

GENERAL ASPECTS OF TISSUE CULTURE FOR COMPOUND PRODUCTION

The primary step in plant tissue culture is to develop a callus tissue from the explant of parent plant in which a callus is defined as a mass of proliferated cells [32]. MPCSC (medicinal plant cell suspension cultures) small group of cells from medicinal plant from callus scattered in a liquid medium for mass production of phytochemicals for pharmaceutical use [33]. Secondary metabolites are derived from plants that play a vital role in human life. A wide range of pharmaceuticals are produced in unorganized callus or suspension cultures which have been succeeded in research till date [34].

Commercialization of suitable metabolites using large scale production through plant tissue culture has driven to a newer technology [35]. In India, the first metabolite produced under *in vitro* conditions is 'reserpine' from *Rauvolfia serpentina* [36]. The production of novel compounds with uniform quality and yield is ensured through tissue culture technology [37]. One of the most effective strategies to improve the production of secondary metabolites is using elicitation [38]. Elicitors are classified as biotic and abiotic. Biotic elicitors are from plant itself and abiotic elicitors are chemical compounds [39]. The first biotic elicitor was discovered in 1968 [40]. Bioproduction of desired natural products is provided with a platform through plant cell suspension cultures as alternative biotechnological methods [41]. These compounds can be produced under *in vitro* conditions that can be used as a therapeutic purpose. Over the last decades, various bioreactors have been developed for tissue development based on different physical stimulation concepts [42]. Bioreactors are devoted to mass propagation of cultivating cells, tissues in liquid suspensions [43]. A bioreactor system has several advantages such as time saving, growth and multiplication and nutrient supply in liquid medium [44].

As snake bite has remained to be one of the unintended cause of deaths in India and also the significance of public health has been misjudged scientifically [17]. So, the investigation of several plant species in this herbal world has given rise to the treatment for most of the ignored diseases across the globe. Some of the plants inhibiting the role of venom using compounds have been explored through literature surveys. Supply of anti-venom is in demand but plants possess the compounds that inhibit the progression of venom and commercial drugs can also be manufactured through tissue culture technology.

In Vitro Production of Compounds against Venom

The leaf discs of *Andrographispaniculata* were inoculated in the MS medium with 1mg/l concentration of NAA and BAP which revealed the best proliferation of callus [45]. Similarly, in our research work, the leaf explants of *E.hirta* was used to develop a callus for defining the optimum concentrations of hormones like 2,4-D, NAA and BAP. The proliferated calli cells were subcultured on MS medium after 3-4 weeks interval with 1mg/ml concentration of NAA and BAP. Likewise the cell suspension cultures of *E hirta* were initiated for the production of triterpenoids in our study. Growth kinetic parameters were evaluated for suspension cultures of *E.hirta* cells. The production of phenolic compounds were enhanced using L-phenylalanine, cinnamic acid, ferulic acid and sinapic acid to the cultures of *Lareeadi varicate* [46]. MJ (Methyl Jasmonate) a proven effective elicitor for the production of taxol in *Taxuschinensis*Roxb [47]. Moreover, in our study the enhancement of triterpenoid production in suspension cultures of *E. hirta* involved addition of biotic (yeast) and abiotic(UV) elicitors during batch cultures which showed elicited amount of triterpenes compared to control using HPLC analysis. *In vitro* production of quercetin can be exploited for large scale production from leaf callus cultures of *Pluchealanceolata* [48]. Thus, the large scale production of triterpenoids (taraxerol) can be explored from leaf calli cultures of *E.hirta* cells that can be used as a drug.

Plants used in Snake Bite Treatment (In Vivo Studies)

The root extract of *Hemidesmusindicus* showed inhibiting activity against PLA₂ using the compound isolated 2-hydroxy-4-methoxy benzoic acid [49]. The aqueous extract of the whole plant have reported to counteract the venom of *Najanaja* [50]. *Theasinensis* and *Cordiaverbenacea* counteracts against the PLA₂ venom efficiently [51, 52]. Several lethal studies reported that *Aristolochiaodoratissima* leafextract showed its activity against *Bothropsatrox* venom at higher doses [53]. Compounds isolated from medicinal plant *Tragiainvolucrata* consists of 2,6-dimethyl heptane, 2,4-dimethyl hexane and 2-methylnonane that belong to hydrocarbon group which revealed the neutralization of PLA₂ venom in mice [4]. The methanolic extract of leaves of *Azadirachtaindica* blocked the PLA₂ enzyme using the isolated compound AIPLAI [54]. Leaf extracts of *Morus alba* showed eradication of *in vitro*proteolytic and hyaluronolytic activities against venom from *Daboiarusselli* [55]. The phenolic content of *Areca catechu* and *Quercusinfectoria* were found to inhibit Phospholipase A₂ activity [56]. The root extracts of *Acoruscalamus* and *Withaniasomnifera* showed in neutralizing effect of PLA₂ activity [57]. Similarly, *E hirta* plant was selected for our study due to the fact which revealed the neutralization of PLA₂ venom due to the presence of triterpenoid compounds with earlier reports. The methanolic leaf extract of *E hirta* revealed neutralizing efficacy of venom [58].

Various Inhibitors against Snake Venom using Insilico Analysis

Inhibitors are developed for further use as compounds or drugs in the treatment of snake envenomation. Inhibitors for PLA₂ play an important role in medicine with the use of some medicinal plants like *Withaniasomnifera* [59]. Various other types of inhibitors are developed to inhibit the PLA₂ activity using *in silico* methods which include curcumin and chlorogenic acid that bind into the active site of the receptor [60, 61]. Molecules extracted from plants include 2-dihydroxy 4-methoxy benzoic acid and 5-hydroxy 7-8 dimethoxy from root of *Hemidesmusindicus* and *Andrographispaniculata* plants respectively [5].The compounds used for docking of *Bungaruscaeruleus* snake venom that showed positive docking score in the active site of PLA₂ are aristolochic acid and lecuasin [62]. In our study the *in silico* analysis revealed that pentacyclic triterpenoid compounds were found to inhibit the PLA₂ proteins of *Naja-naja*, *Bungaruscaerulus*, *Echiscaranatus* and *Daboiarusselli* by showing positive docking score and amino acid residues interacting in the active

site. The compounds included taraxerol, oleanolic acid, taraxasterol that can be produced using tissue culture as a drug in near future.

CONCLUSIONS

Snakes are found in enormous numbers belonging to different group of families with venom causing deaths across the world. From this survey it reveals that deaths caused due to envenomation are ruthless and treatment is far behind. Development of herbal formulation for the treatment of snake bite has become one of the challenging treatment. This is my unpretentious effort to make a survey on medicinal plants amidst all the contributions from the researchers towards treatment of snake bite with *in vitro* and *in vivo* studies using herbal plants. Discovery of new drugs (inhibitors) through *in silico* analysis has confirmed the activity against the venom. Large scale production of these metabolites plays an important role in the bioavailability, cost effective and treatment using these phytochemicals from plants through tissue culture technology.

FUTURE PROSPECTS

Treatment of snake bite remains still unclear even today. Earlier, anti-venom from immune horse's serum, immunotherapy, etc was used. Nowadays, Medicinal plants are in tremendous use for the production of secondary metabolites using *in vitro* studies and it's activity against the venom for inhibition is done using *in vivo* studies. Application of these drugs can be produced in large scale based on *in silico* studies with that of the toxic enzymes present in the venom. Further these compounds identified can be produced as commercial drugs in near future. The effect of some toxins like PLA₂ present in all snakes can be a competent inhibitor by using these novel methods of envenomation.

AUTHOR'S CONTRIBUTION

Gandepalli Pratap Kumar: Compiled the entire literature search and drafted the manuscript. Beena Kanimozhi R: Reviewed and edited the manuscript and developed according to the standard. R. S. David Paul Raj: Supervisor of the research work, provided guidance in the preparation of the manuscript and also conceptualized the article.

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CONFLICTS OF INTERESTS

Authors declared no conflicts of interests.

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