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Natural Products Such as RIPs in living organisms

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ABSTRACT
Ribosome-inactivating proteins have been discovered in bacteria, fungi, algae, and plants (RIPs). Because of N-glycosylase activity, RIPs cleavage adenine residues at a conserved site on the 28S rRNA.The cleavage of this single N-glycoside bond inhibits protein synthesis because it interferes with the elongation factors' ability to associate with the ribosome. MAP30 is an anti-HIV plant protein from bitter melon and has anti-tumor properties, as well as topological inactivation of viral DNA, suppression of viral integrase, and cell-free ribosome inactivation. Riproximin was used to treat colorectal cancer. Riproximin is isolated from Ximenia Americana which is a type 2 RIP. MAP30 has more therapeutic potential than other RIPs since it is not only effective against HSV and HIV infection and replication, but it is also nontoxic to normal cells. Ribosome-inactivating proteins to obtain resistance against fungal pathogens are based on the ability of some RIPs to depurinate the ribosome of various fungi. Soybean toxin (SBTX) is a toxin produced by soybeans that is harmful to pathogenic fungi and yeast.
Keywords: *Ribosome-inactivating protein; cancer immunotherapy; viral integrase; Riproximin, SBTX, rRNA N-glycosylase.*

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INTRODUCTION

Plants, fungi, algae, and bacteria have ribosome-inactivating proteins (RIPs). Cleavage of this single N-glycosidic bond is irreversible and interferes with the association between the elongation factors and ribosome, causing the inhibition of protein synthesis [1]. RIPs were first discovered in plants, primarily in the Angiopermae family (monocotyledons and dicotyledons), as well as mushrooms and the algae *Laminaria japonica*[2],[3].

Based on their physical characteristics, RIPs are divided into three categories. Type 1 RIPs are single-chain proteins with a molecular weight of about 30-kDa and a high basic (pI typically >9.5); the majority are glycoproteins. Type 2 RIPs are a toxin that consists of two polypeptide chains: an enzymatically active A chain and a lectin-binding B chain. The A chain is responsible for the toxic activity of the protein, while the B chain facilitates the binding of the protein to cells. Type 2 RIPs A chain molecular weight of typically around 30kDa (pI between 4.8 and 8), while the molecular weight of the B chain is typically around 34 kDa (pI~7) [4]. Type 3 RIPs are characterized by the presence of an additional domain at the C-terminus of the RIP domain. This domain is not essential for the RIP activity, but its function is unknown. Two types 3 RIPs have been characterized, barley JIP60 and maize b-32. Barley 60 has a molecular weight of 60 kDa and an isoelectric point of 9.4. It is synthesized as a single-domain proenzyme that is activated by the removal of a 12-residue single peptide. Maize b-32 has a molecular weight of 34 kDa and an isoelectric point of 9.2 (Fig.1) [5], [6].

Newly identified ribosome-inactivating proteins (RIPs) are found in various organisms. These include RIPs Anopheles and Culex mosquitoes (Mosquito RIPs), antimicrobial peptides and plant RIPs. Plants in the Cucurbitaceae family, such as bitter gourd (momorcharin), bottle gourd (lagenin), and pumpkin (cucumoschin), produce smaller RIPs with different molecular weights compared to regular type 1 RIPs. Other plants such as camphor tree seeds (cinnamomin), snake gourd (trichokirin), golden needle mushroom (flammulin), mosaic puffball mushroom (calcaelin), as well as bacteria (Shiga toxin), and *Streptomyces coelicolor* Produces RIP. These proteins exhibit diverse molecular weights, amino acid sequences, mechanisms of action, and possess valuable antipathogenic properties [7].

Maize b-32 is a type 3 RIP that is synthesized as a 30-kDa precursor that requires proteolytic removal of an intrinsic peptide for activation. As a result, A chain of roughly 17 kDa and a B chain of approximately 9 kDa are produced. Both of these chains are necessary for N-glycosidase activity, unlike Type 2 RIPs [8], [9]. The

physiological effects of RIPs on mammalian cells have been linked to apoptotic pathways. Antiviral, antifungal, and insecticidal activities shown in vitro and transgenic plants have linked RIPs to plant defense [8]. Based on the structure of the A-B toxins, RIP has been regarded as a prototype for the production of chimeric molecules known as immunotoxins [10]. Both normal and tumor cells are suppressed by RIP (Saporin), which inhibits protein synthesis and proliferation. RIP (Saporin) induces cell death [11]. Furthermore, the use of inhibitors and pharmacological agents that disrupt signal transduction pathways leading to programmed cell death adds to the evidence that RIPs trigger apoptosis [8]. Ledodin is a 22-kDa protein isolated from mushrooms (*Lentinula edodes*) that exhibit N- glycosylase activity and inhibits protein synthesis, but it does not affect insect, bacterial, or fungal ribosomes. It is the first of a new family of enzymes widely distributed among this class of basidiomycetes [12].

Occurrence of Ribosome-inactivating Proteins

RIPs have been isolated from 50 different plant species belonging to 17 different families [13]. RIPs are widely found in plants of Angiosperms such as Monocots, Eudicots, and Magnolidis. RIPs gene has been identified in orders Poales, Laurales, Asparagales, Arecales, Fabales Cucurbitales, Rosales, Malphigiales, Dipscales, Caryophllales, Legname, and Santales [14]. Except for Spermatophyta, no A domain could be identified in any members of the Anthocerotophyta, Bryophyta, Marchantiophyta, or Euphyllophyta [15]. The higher conc. of RIPs was found in the seeds of Caryophyllaceae, Cucurbitacea, Euphorbiaceae, and Phytolaccaceae [4].

Types of Ribosome-Inactivating Proteins

RIPs from plants have been divided into three main types based on their physical properties, including type I, type II and type III.

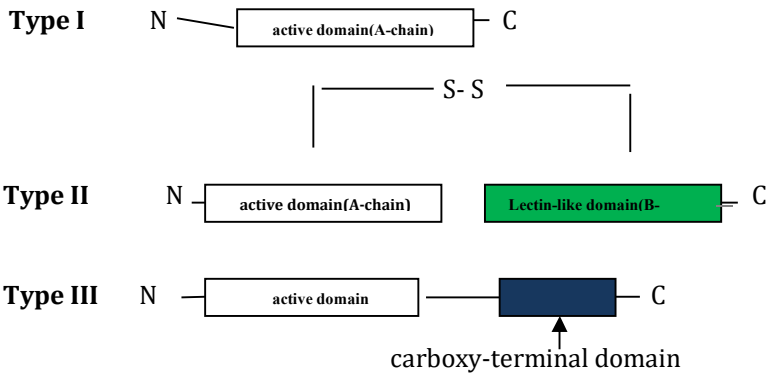
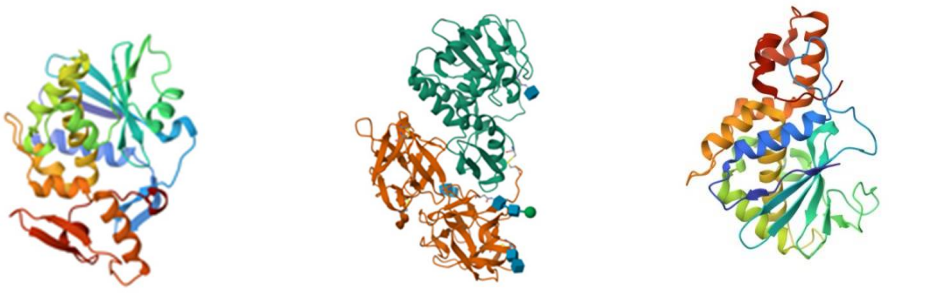


Fig.1- Schematic representation of the mature form of three types of ribosome-inactivating proteins.



(a) (b) (c)

(a) Bryodin Protein Type I RIP

(b) Mistletoe Protein Type II RIP

(c) Maize b-32 Protein Type III RIP

Fig. 2- Folding pattern of RIP

Molecular structure of Bryodin (1BRY), Mistletoe (1PC8), and Maize b-32 (2PQI) Type I RIP Bryodin contains a single N-glycosidase catalytic A domain: type II RIP Mistletoe is made of two chains linked by an S-S bond displaying a galactose binding domain (B chain) and A chain catalytic active domain: Type III RIP Maize b-32 is a two-chain protein, consisting of an A chain and a B chain. The A chain is the catalytic subunit of the protein, and B chain is corboxy-terminal domain.

Type - I RIP

Type-I ribosome-inactivating proteins are distributed in Asparagaceae, Caryophyllaceae, Cucurbitaceae, Euphorbiaceae, Nyctaginaceae, Phytolacaceae, and Poaceae family. Type-1 RIPs such as Bryodin purified from *Bryonia dioica L* are Bryodin-L mol. wt 28.80kDa, Bryodin-R mol. wt 30.00kDa (Fig.2a) [16],[17]. Type-I proteins isolated from *Agrostemma githago* are agrostin 2 (pI= 7.7) mol. wt 29.2 kDa, agrostin 5 (pI =8.7) mol. wt 25.5kDa, and agrostin 6 (pI =8.75) mol.wt 27kDa. Three proteins saporin 5, saporin 6 (pI9.5) mol.wt 29kDa, and saporin 9 (pI 9.5) mol.wt. 27 kDa have been identified from *Saphonaria officinalis* (carnation) seeds (RIP type 1 protein) [18]. Two proteins, dianthin 30 (mol. wt 29.5 kDa) and dianthin 32 (mol. wt. 31.7 kDa), were isolated from *Dianthus caryophyus* (carnation) leaves. These proteins have antiviral and ribosome-damaging activities [19]. The isolated protein luffin (mol. wt. 26kDa) isolated in the seeds of *Luffa cylindria roem* inhibits protein synthesis. This protein has a significant inhibitory effect on protein synthesis. This activity is about 10 times as strong as that of the ricin A-chain [20]. Gelonin has been isolated from the seed of *Gelonium multifluorum*. These proteins' molecular weight is approximately 30KDa [21]. The barley toxin is a single polypeptide with a molecular weight of approximately 30kDa that is toxic to animal cells [22]. PAP is a type I RIP from the leaves of *Phytolacca Americana* with a molecular weight of 27 to 30kDa [23].

Type-2 RIP

Types-2 RIPs occur in certain families like Lauraceae, Ranunnculaceae, Passifloraceae, Cucurbitaceae, Fabaceae, Viscaceae, Euphorbiaceae, and Caprifoliaceae [24]. In a cell-free system, ricin, a very poisonous protein present in castor beans (*Ricinus communis L.*), inhibits protein synthesis. Ricin is made up of two peptide chains joined by disulfide bonds. The molecular weights of the two peptide chains were calculated to be 32kDa and 34kDa, respectively. Ricin has two component chains (A and B) that are capable of inhibiting protein synthesis, with the A chain being poisonous and the B chain being nontoxic [25]. Although an extract of the *Viscum album* known as Iscador is commonly used in the treatment of cancer, there is no clear evidence that it has antitumor properties. *Viscumin* is a toxin extracted from the *viscumin album L*. (mistletoe). There is a mol. wt of 60kDa there. It is made up of two disulfide-bonded peptide chains, chain A having mol. wt. 29kDa and chain B having mol. wt. 32kDa (Fig.2b) [26], [27]. Kirkiin is a newly discovered type2 ribosome-inactivating protein (RIP) found in the caudex of *Adenia Kirkii*, which is a galactose-binding lectin capable of efficiently inhibiting protein synthesis and agglutinating erythrocytes. Additionally, kirkiin exhibits biochemical, enzymatic, and cytotoxic properties. These RIPs possess N-glycosylase activity on mammalian and yeast ribosomes but show little or no activity on other nucleotide substrates. This can completely inhibit cell protein synthesis and induce cell death through apoptosis at extremely low doses [28]. Cinnamomin is a type 2 RIP from the seeds of *Cinnamomum camphora* having a molecular weight of 61kDa [29].

Type-3 RIP

The Opaque-2 regulatory site has been found to regulate the synthesis of the cytosolic *maize albumin* b-32. It has been shown that b-32 is a hazardous plant protein with ribosome-inactivating action that belongs to a broad and extensively dispersed class (Fig.2c)) [30],[31]. RIP is synthesized and stored as a 34kDa inactive precursor (pI=6.5) in the kernel of maize (*Zea mays*). This neutral precursor is changed into a basic active form by proteolysis which removes 25 amino acids during germination. (2.8kDa) of net charge-6 from the polypeptide chain's center [5]. JIP60 is a ribosome-inactivating protein (RIP) identified from barley. JIP60 is a methyl jasmonate-induced ribosome-inactivating protein (JIP60) implicated in plant stress responses. In rabbit reticulocyte lysates, JIP 60 decreases the rate of in vitro translation of plant mRNAs [32].

Pharmacology of RIP

RIP with antitumor activity

Type I RIP

Luffin is a RIP derived from *Luffa cylindrica L*. seeds (sponge gourd). In a cell-free translation system, Luffa-a and Luffa-b have inhibitory effects on protein synthesis [33]. MAP 30, an anti-HIV plant protein was isolated from bitter melon (Momordica charantia). It can act at many stages of the viral life cycle, acute infection, and replication in chronically infected cells. MAP 30 also has anti-tumor properties, as well as topological inactivation of viral DNA, suppression of viral integrase, and cell-free ribosome inactivation [34]. The effects of the recombinant protein on cell proliferation and apoptosis in human colorectal cancer LoVo cells were studied after MAP30 was cloned and expressed [35]. MCP30, also known as α-momorcharin and β-momorcharin, is a single-chain Type-I ribosome-inactivating protein (RIP). MCP30 causes apoptosis in PIN and Pca cell lines *in vitro* and reduces PC-3 growth in vivo while having no impact on normal prostate cells. MCP 30 inhibits the activity of histone deacetylase-1 (HDAC-1) and increases the acetylation of histone-3 and histone-4 proteins [36. *M. charantia* RIPs have been shown to have anti-tumor activity both *in vitro* and *in vivo*. MAP30 was shown to have anti-tumor properties. Certain human tumor

cell lines from renal, and breast cancer showed anti-tumor activity when exposed to MAP30 [37]. Dianthin-30 is very similar to two other ribosome-inactivating proteins (RIPS), namely saporin-S3 and saporin-S6. These two RIPs are commonly utilized in the development of targeted toxins for tumor therapy and have been subjected to clinical trials. But, dianthin enzymes also have well-known distinctions from saporins in terms of their structure, efficacy, immunogenicity, toxicity, and other activity. Some of these differences might render dianthin more suitable for targeted tumor therapies compared to other RIPs. Initial investigations conducted on mice, utilizing targeted dianthin in the presence of endosomal escape enhancers, revealed the immense potential of this plant enzyme [38]. Recombinant OsRIP a type 1 RIP from (*Oryza sativa L.*), was examined for its anti-proliferation effects in this work, which led to plasma membrane blebbing resembling apoptosis without DNA fragmentation. Potential interaction partners for OsRIP1 include (the Apoptosis-Stimulating Protein of p53 1) and IFITM3 (Interferon-Induced Transmembrane Protein 3) [39]. Quinonin, a novel type 1 RIP isolated from quinoa seeds. Quinoin could represent a novel tool for glioblastoma (cancer) therapy and a possible adjuvant for the treatment of the disease in combination with temozolomide (TMZ) [40].

Type II RIPs

Ebulin is a type 2 ribosome-inactivating protein found in the *Sambucus ebulus* L. plant (Caprifoliaceae). *In vitro*, ebulin-I inhibited protein synthesis in rabbit reticulocyte lysates, rat brain, and rat liver cell-free systems, but did not affect protein synthesis in plants or bacteria. Cervical epithelioid cancer was treated with it [41],[3]. Mistletoe is a protein that prevents ribosomes from being activated. *Viscum album* produced this protein. The active principle of extensively used in mistletoe treatment in Europe for anticancer and immunomodulatory effects. This protein has been approved by the FDA for use in clinical trials and exhibits immunomodulatory properties [42]. RIPs can be used to make conjugates directed to a specific target, such as the overexpressed transferrin receptor (TFR) in cancer cells. TFR-overexpressing cancer cells are conjugated in this way [43]. The recently found protein, riproximin, belongs to the type II ribosome-inactivating protein family and was isolated from the *Ximenia Amenia americana*. In African traditional medicine, *Ximenia Americana* plants are used to cure cancer. Riproximin's potential to suppress protein synthesis in a cell-free system, as well as riproximin's cytotoxicity. Riproximin was used to treat colorectal cancer. Riproximin is a new type II ribosome-inactivating protein that is isolated from *Ximenia americana*. This compound was used in African traditional medicine and exerts highly impotent anticancer activity in vitro and vivo [44].

RIP with antiviral activity

The antiviral properties of RIPs have been studied for over 4 decades. However, interest in these proteins is growing because of the emergence of infectious diseases caused by new viruses and the difficulty of treating viral infections. Phytosanitary products are very harmful to the environment and in this respect, RIPs have been shown as a promising tool that can be used to obtain transgenic plants resistant to viruses [45]. In nature, products from various sources undoubtedly play an important role in the treatment or regulation of biochemical pathways involved in the progression of the disease. RIPs can catalytically inactivate not only eukaryotic but also prokaryotic ribosomes by preventing protein production. However, some vaccines have been fully approved by the FDA, so the end of the global pandemic is now, but COVID-19 is still the leading cause of death worldwide with a decreasing rate. rRNA depurination affects protein synthesis and induces a ribotoxic stress response that initiates apoptosis cascades. The ribotoxic stress response regulates the transcription mechanism and expression level of many other proteins by activating MAPK in the cell, suggesting the RIPs may be a key enzyme in the expression of targeted viral proteins [46]. MAP30 (Momordica Anti-HIV Protein), α- and β-momorcharins all suppress HIV replication in chronically infected cells, making them promising therapeutic agents for HIV infection and AIDS. Furthermore, when combined with other antiviral drugs, MAP30 enhanced the effectiveness of anti-HIV therapy. MAP30 has more therapeutic potential than other RIPs since it is not only effective against HSV and HIV infection and replication, but it is also nontoxic to normal cells [37]. Clinical responses to Trichosanthin were monitored largely by increases in CD4+ T cell counts in patients with AIDS who had failed to respond to antiretroviral drugs like zidovudine. Trichosanthin-treated cells had low amounts of p24 antigen but large quantities of p24 antibodies [47],[3]. Luffin P1, the smallest ribosome-inactivating peptide extracted from *Luffa cylindrical* seeds, and anti-HIV-1 activity was discovered. Lentiviruses, such as the *Human Immunodeficiency Virus* (HIV), require the Rev-responsive element (RRE) for replication (HIV-1). The viral transacting regulatory protein is bound by the Rev-responsive element (RRE) [48],[49].

Application of RIPs in gene therapy

Toxic gene therapy, sometimes known as suicidal gene therapy, was developed after the successful preparation of two mammalian transfection vectors, one expressing the gelonin gene and the other having the saporin gene. These RIP gene constructs were successfully transfected into a variety of tumor cells and showed high cytotoxicity. With the use of appropriate transfection vehicles, these plasmids may be used as

anticancer drug possibilities due to their high efficiency in killing tumor cells [50]. This is the first time that researchers have successfully produced a large amount of recombinant gelonin (rGel) in *E.coli* BL21 bacteria and its antitumor activity was evaluated in two colon cancer cell lines[51]. Cytotoxic proteins (Ribosome inactivating proteins, RIPs) can effectively retain inside the cells and prevent drug efflux mediated by multidrug resistance transporters due to the large-size effect [52]. The pSERPINB3-PE38KDEL plasmid might be a hopeful approach for targeting OSCC gene therapy [53]. Suicide gene therapy is a relatively novel form of cancer therapy. Some RIPs from bacteria and plants have been studied to see how they can be delivered and controlled in tumor cells [54].

Immunotoxins

Various toxins, such as Gelonin, PAP, Saporin-6, ricin, or its A chain, have been conjugated to antibodies, mostly monoclonal, to produce immunotoxins that are toxic only to the antibody's target cells [4]. Ricin and abrin proteins can be used as chemical weapons but are primarily used in medicine to create immunotoxins that target tumor cells. Their most obvious use, in this case, is in aerosols, which would result in lethal lung damage [12]. Immunotoxins are beneficial in cancer and parasite disease treatment. Murine monoclonal antibodies covalently linked to ricin were used. Anti-T cell Immunotoxins are used to treat donor bone marrow ex vivo to prevent Graft- Versus- Host Disease [55]. Gelonin is an inhibitor of protein synthesis by eukaryotic ribosomes, similar to the A chain of abrin and ricin. Thy1.1-bearing AKR-T cell lines were killed by anti-Thy1.1 gelonin conjugates [56]. Acute lymphoblastic leukemia cells were effectively killed by immunotoxins containing pokeweed antiviral protein and monoclonal antibodies targeting human T-cells or human transferrin receptors [57]. Pokeweed Antiviral Protein (PAP), a human B-cell-specific immunotoxin, has improved its ability to selectively eliminate B-cells. The immunotoxin's monoclonal antibody (B43) was directed toward human B-cells and had a disulfide linkage with the pokeweed antiviral protein [58]. Saporin inhibits protein synthesis in reticulocyte lysates by an efficient RIP. The immunotoxin produced by covalently linking the RIP saporin to a monoclonal anti-Thy1.1 antibody was strong and specific for Thy 1.1 expressing cells in tissue culture in animals. It has antitumor properties both *in vitro* and *in vivo* [59].

Effects of RIPs on the immune system

Gelonin is an immunologically active protein that functions as a protein synthesis inhibitor in a similar way to the Ricin-A chain. It was examined in mice to see if it may be useful in the development of antibody-toxin conjugates. *In vivo,* the treatment of Gelonin inhibited macrophagic (but not NK) cytotoxicity and mitogen responses. Gelonin reduced initial responses to a T-dependent and, to a lesser extent, a T-independent antigen when given before stimulation, as well as resistance to allogeneic tumor grafts and L. Monocytogenes challenges [60]. *Momordica Charantia inhibitor* (MCI) and Pokeweed antiviral protein (PAP-S), which have a closer similarity to the Ricin-A chain, have been shown to suppress protein synthesis in mice. H2-incompatible skin allograft rejection, splenocyte response to Con A, and PHA were all slowed by these proteins. This substance could also lessen NK cell activity at the same time as growing macrophage-mediated spontaneous cytotoxicity [61].

Development of natural Bio-pesticides

Antibacterial, antifungal, and antiviral properties have been reported in many RIPs. ME1 and ME2 have identified the root of *Mirabilis expansa*. *Pseudomonas syringae*, *Agrobacterium tumefaciens*, and *Agrobacterium radiobacter* were all found to be resistant to the ME1 and ME2 RIP [62]. Genetic engineering is the most common method used to introduce resistance in plants against various pathogens. RIPs, which are rRNA N-glycosylases, inhibit translation by enzymatically inactivating ribosomes. They are classified into three types: Type I RIPs consist of a single catalytic A chain, while Type II RIPs are made up of two chains. Type III RIPs include proteins that are induced by Jasmonic acid. Certain RIPs have been expressed through genetic recombination and shown to have catalytic activity. Plants that carry RIP transgene exhibit resistance to viruses, fungi, and insects [63].

Role of Ribosome-Inactivating Proteins against Fungi

RIP plays an important role in the plant's defense mechanism against bacteria, fungi and even plant-eating animals [64]. Ribosome-inactivating proteins to obtain resistance against fungal pathogens is based on the ability of some RIPs to depurinate ribosomes of used various fungi [65]. Soybean toxin (SBTX) is a toxin produced by soybeans that is harmful to pathogenic fungi and yeast. RIP interferes with an intracellular proton transfer to the external media and inhibits spore germination. Cell wall breakdown, cytosol condensation/shrinkage, pseudohyphae formation, and cell death were all caused by RIP [66]. α -sarcin, a type-1 RIP, cleaves one of the 28S rRNA's phosphodiester bonds. Other antifungal proteins have been shown to have synergistic effects with RIP. The N-glycosidase activity of barley RIPs inhibits protein synthesis. Antifungal activity is found in hairy melon RIP and small RIP luffacylin from *Luffa cylindrical* seeds [67]. The growth of the soil-borne fungus was inhibited by root exudates containing PAP-H and additional chitinase, -1-3 glucanase, and protease activities. *In vitro* and *in vivo*, PAP-H depurinates fungal

ribosomes, suggesting that PAP-H enters fungal cells by an additive mechanism [68]. *Fusarium solani* and *Fusarium oxysporum* mycelia growth are inhibited by alpha-momorcharin (α-MC). It inhibits *Pseudomonas aeruginosa* growth and induces apoptosis in *F. solani* [69].

The Significant Role of RIPs in Insects

Ribosome-inactivating proteins (RIPs) are toxic proteins with N- glycosidase activity that inhibits protein synthesis. They can target both prokaryotic and eukaryotic cells and have been engineered to target specific cell types. In bacterial RIPs help in the process of pathogenesis while in plants RIPs have been to aid defense against insect, viral, bacterial, and fungal pathogens [70]. Plants with induced expression of RIPs have been found to have enhanced resistance to viruses, fungi, insects, drought, and salt [71]. Many insects have extensive symbiotic bacteria relationships, and the insect symbionts frequently play critical roles in the host's survival, such as supplementing nutrition and even giving protection from natural predators [3]. RIPs have a strong aphicidal activity against pea aphids. Significant sublethal effects were also found in the surviving aphids, with a reduction in fecundity, intrinsic rate of increase, net reproductive rate, and population doubling time [72]. Corn earworm, *Helicoverpa zea*, resistance was found in transgenic tobacco (*Nicotiana tabacum L.*) lines expressing an activated form of maize (*Zea mays L.*) ribosome-inactivating protein (RIP) [73]. Transgenic tobacco (*Nicotiana tobacum*) plants expressing activated maize ribosome-inactivating protein (RIP) and a line overexpressing tobacco anionic peroxide were compared to the wild-type cross for their effects on corn earworm *Helicoverpa zea* and cigarette beetle *Lasioderma serricorne* larve. For those insects, there was a significant reduction in feeding and an increase in death [74]. SNA-I Protein was obtained from *Sambucus nigra*. SNA-I is a RIP that belongs to the Type-2 RIP class. Two pest insects, *Acyrthosiphon pisum* and *Spodoptera exigua*, died as a result of this RIP. Through induction of caspase-3-like activity, this protein has been involved in cell death or apoptosis in the entomotoxicity of SNA-I [75].

Future Perspectives

RIPs are stimulating interest in their biological roles in plants after a year of being researched as tools to be utilized for their toxicity. Transgenic plant technologies have opened up new possibilities for targeting RIPs to new locations inside plants, expressing RIPs in plants that lack endogenous RIP activity, and inactivating endogenous RIP genes to see just how far they affect plant phenotypes [8]. All of the RIPs that have been studied so far show antiviral effects against plant or animal viruses but do not act against viruses that harm their species [4]. The use of green fluorescent RIP fusions in the investigation of internalized was studied in peptides retrograde transport of cells. The novel technology, photochemical internalization (PCI), was discovered to efficiently deliver type-I ribosome-inactivating proteins encoding green fluorescent protein into the cytosol in a light-dependent manner. PCI induces efficient light-directed delivery of macromolecules into the cytosol which is useful in drug delivery such as in cancer treatment, vaccination, and gene therapy [76]. Since hydrolytic enzymes in lysosomes have been discovered to reduce the cytotoxic impact of gelonin on cancer cells, macromolecules can be imported through PCI from both pre-lysosomal and lysosomal compartments into the cytosol. RIPs are a protein that acts against HIV and cancer. An RNA and DNA glycosylase activity, as well as a DNA apurinic/apyrimidinic (AP) lyase, has been identified [77]. *In silico* analysis was done to know the molecular and structural properties of Ribosome inactivating protein (tritin protein) obtained from common wheat plants (*Triticum aestivum L.*) [78]. Ribosome-inactivating proteins (RIPs) are potential transgenes that can be used to create genetically modified plants to fight against different environmental stresses, cause ectopic expression of pathogenesis-related proteins, and trigger systemic acquired resistance. These genes are activated when specific environmental signals are present, which helps the transgenic plants avoid extra stress on their systems [79].

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