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REVIEW ARTICLE

Toxicity of Biologically Active Peptides and Future Safety Aspects: An Update

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Abstract: ***Introduction:*** Peptides are fragments of proteins with significant biological activities. These peptides are encoded in the protein sequence. Initially, such peptides are inactive in their parental form, unless proteolytic enzymes are released. These peptides exhibit various functions and play a therapeutic role in the body.

Objective: Besides the therapeutic and physiological activities of peptides, the main purpose of this study was to highlight the safety aspects of peptides.

Method: We performed an organized toxicity and search of available literature using PubMed, Google Scholar, Medline, EMBASE, Reaxys and Scopus databases. All the relevant citations including research and review articles about the toxicity of biologically active peptides were evaluated and gathered in this study.

Results: Biological peptides are widely used in the daily routine ranging from food production to the cosmetics industry and also they have a beneficial role in the treatment and prevention of different diseases. These peptides are manufactured by both chemical and biotechnological techniques, which show negligible toxicity, however, some naturally occurring peptides and enzymes may induce high toxicity. Depending upon the demand and expected use in the food or pharmaceutical industry, we need different approaches to acertain the safety of these peptides preferentially through *in silico* methods.

Conclusion: Intestinal wall disruption, erythrocytes and lymphocytes toxicity, free radical production, enzymopathic and immunopathic tissue damage and cytotoxicity due to the consumption of peptides are the main problems in the biological system that lead to various complicated disorders. Therefore, before considering biologically active peptides for food production and for therapeutic purpose, it is first necessary to evaluate the immunogenicity and toxicities of peptides.

Keywords: Peptides, Safety, Prevention, disease, *In silico,* Computational biology.

1. INTRODUCTION

Proteins are important constituents of the food that play a vital role in the regulation of different body functions. It is a good source of biologically

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active peptides. The biologically active peptides can be defined as, those peptides which interact with various body receptors, leading to effects that are either beneficial or toxic. These peptides are very important as they mostly act as angiotensin-converting enzymes. There are different peptides present in the foods most of them can be used in the prevention of chronic diseases [1].

In the living organisms, during the digestion of food in the gastrointestinal tract, small peptides are continuously released which act as regulatory compounds and play an important physiological role in the body. These peptides are derived from proteins and these proteins are the source of biologically active peptides which can be obtained from various foods such as milk, plants and meat. The peptides derived from proteins are beneficial for the regulation of different body functions such as maintaining blood pressure and stimulation of the immune system [2]. Besides their physiological role, these peptides act as antibacterial and antioxidant in the body [3]. The application of biological peptides for the purpose to cure certain diseases such as cancer and immunological disorders is an area of interest for various research groups. The main aim of this review was to focus on the toxicity of biologically active peptides and associated future safety aspects.

2. TOXICITY OF BIOLOGICAL PEPTIDES

Peptides toxicity is the cause of celiac disease, a serious genetic autoimmune disorder produced by the ingestion of gluten protein leading to the damage to the intestinal walls. Gluten proteins are found in a variety of foods such as wheat, barley and rye [4]. It is a serious global problem and approximately one person among hundred individuals is affected. In order to treat this disease, gluten-free diet is used. Furthermore, oral medicines in the form of tablets and capsules may have ingredients composed of gluten. Most of the naturally occurring peptides and enzymes are potentially toxic to the unicellular and multicellular organisms, which can be found in the plants, animal by-products and even in dried food products (Table **1**). There are two hypotheses regarding the development of celiac disease. One is the enzymopathic hypothesis while the other is the immunological. In the enzymopathic hypothesis, it is assumed that incomplete proteolysis of the peptides molecules causes an abrupt increase in the concentration of these peptides in the large intestine and consequently damage tissues. While in case of immunological hypothesis, the fragments of the gluten proteins bind to the intestinal membrane and initiate immunological reactions [5]. The specific features of the celiac toxic peptides are the high amount of glutamine, proline and tyrosine. The examples of proline-rich peptides are 12-mer, 19-mer and 33-mer, respectively. These peptides are produced by the action of proteolytic enzymes and hence it is toxic, especially for celiac patients, but not for all healthy individuals [6].

It has been reported that certain endogenous prolyl oligopeptides play a vital role in the spreading of celiac disease. There are some peptides such as alpha-gliadin, which can be digested by the action of enterocytes both in celiac patients and in normal individuals. As a result of such digestion, the patients have a high amount of incomplete degraded 33-mer peptides. The prolyl endopeptidases' activity inside the mucosa of the duodenum was observed to be much higher in those patients with celiac treatment as compared to the normal subject [7].

In a study, the effect of whey protein digestion on the propagation of lymphocytes was evaluated which showed that these proteins enhanced the production of the lymphocytes whereas no effect on the lymphocytes have been observed for other peptides such as beta-lactoglobulin and alpha-lactalbumin [8].

Beta-amyloid protein (Aβ), is a member of the small group of proteins that is stored in body tissues. It has been revealed that the toxic effects of Aβ on the neural cells is due to the damage associated with oxidative reactions. All the diseases linked to amyloid are characterized by the abnormal depositions of peptides [9]. It is important to note that there is a key relationship between the accumulation of these peptides and human amyloidosis such as amylin and calcitonin, all of which are toxic peptides for the primary cells as well as for clonal cells [10]. The toxicity associated with these peptides is facilitated through the production of the free radical pathways. It has been confirmed by experiments that the toxicity caused by these peptides is due to their amphiphilic nature. The example of amyloidogenic peptide is calcitonin, which is amphiphilic in nature [11].

Table 1. Naturally occurring enzymes and peptides with possible toxicities.

Names	Toxic effects	Origin	References
Hemagglutinins	Clump or agglutinate RBCs, stimulating effect on mitosis of cultured human leukocytes, DNA, RNA and protein synthesis stimulation, immunosuppression, protein-carbohydrate interaction, bind to the specific receptor site in erythrocytes and other cells, bind with cancer cells, enlarged thyroid gland, hypoglycemia	Bananas, potatoes, mangoes, bean and wheat germ plants, fish eggs, snails, sponges,	[20-30]
Gluten	Celiac disease, small or absent villa of jejunum biopsy sample, impaired amino acids, glucose, vitamins-K, B_{12}, disaccharidase and peptidase deficiency, intestinal lesions	Wheat products, gluten-products	[31-33]
Thiamine destroying enzymes or thiaminases	Decomposition or breakdown of vitamins, liberate metabolites	Bracken fern, *Pteridum aquallinum,*	[34]
Lipoxygenase	Oxidized and destroys carotene, lower blood vitamin-A and carotene in the liver and/or blood of calves	Soyabean	[35]
Urease	Liberate ammonia from blood urea,	Jack bean	[36]
Cyanogenetic glucosides	Produce hydrocyanic acid	Plants products soaked and crushed in water	[37]
Intestinal β-glucosidase	Release toxic methylazoxymethanol from the glucoside cycasin	Present in cycad family plant	[38]
Trypsin inhibitor	Retard growth	Soyabean	[39]
Islanditoxin	Hepatoxicity due to the presence of β-aminophenylalanine, two serine, aminobutyric acid, dichlorinated proline, liver cirrhosis	Yellow rice infected with *penicillium islandicum* mold	[40]
Pallotoxins	Cyclic heptapeptides in nature, hepatotoxic, marked affinity microsomal fraction of the liver,	Mushroom	[41-43]
Amatoxins	Closely related to cyclic octapeptides, hepatotoxic, liver nuclei affected	Mushroom	[41]

The active peptides and proteins are circulating in the body by crossing the blood-brain barrier (BBB) through diffusion and/or active transport mechanism across the endothelial cells [12-15]. Peptides and proteins such as TNF, NF-kappa-B-mediated GP-130 and LIFR affect endothelial cells leading to cytotoxicity and altered cell proliferation [16-19]. Several peptides and proteins such as neurotrophic peptides, larger neurotrophins, and cytokines cross the BBB and alter physiological functions of the body.

As shown in the Table **1**, in the last decade, the therapeutic applications of the biologically active peptides have been increased and therefore, their toxic effects should be taken into account. Recently, the advancement in biotechnological techniques revealed certain chemical modifications and alteration in the physicochemical activities of peptides without changing the functions and therapeutics activities of peptides. Several techniques such as lactate dehydrogenase leakage, 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide, ATP-based and hemolytic assays have been used to evaluate the natural and bioactive peptides toxicities. There are various types of webservers which are designed to screen the toxicity of peptides such as clanTox, BTXpred, NTX-pred, VICpred, DBETHA and ToxinPred. For instance, ToxinPred can scan whole proteins for toxicity estimation along with peptides and enzymes [44]. It is important to monitor the toxicity of natural, synthetic and semi-synthetic peptides and proteins, because it attenuates the unwanted noxious effects via genetic mutations in amino acids as shown in the Fig. **1**. Peptides have high potential for penetration, low-cost production, high specificity and biological activities [45]. But, they have low stability along with immunogenicity and toxicity which is the main problem in the production of peptides-based drugs. Peptides containing a combined sequence of α-aminoxy acids and α-amino acids have comparatively good metabolic stability as compared to peptides, consisting of only α-amino acids. The incorporation of an α-aminoxy acid into the existing peptide chain will significantly enhance the stability of the amide bonds. Those peptides containing a mixed structure of α-aminoxy acids and α-amino acids have the ability to become a perfect scaffold for the de novo design of metabolically stable and biologically active peptides [46]. There are, some metals such as cadmium (II) that can also form complexes with amino acids and peptides [47]. But toxicity and oxidative stress issues should be considered while focusing on the those metals that form complexes with aminoacids and peptides [48]. It is evident, that the present *in silico* tools help to predict immunogenicity of peptides [49-52]. The application of computational chemistry techniques is helpful for reducing toxicity and enhancing the functionality of peptides [53]. In a similar study, a multilayer coating containing peptide possessing antimicrobial property was fabricated on smooth titanium through the process of the layer by layer assembly. The AMPCol-loaded layers formed a thin hydrophilic film. The smooth surface enabled the cellular attachment with low levels of cytotoxicity or red blood cells breakdown. A controlled release assay and antimicrobial screening indicated that titanium plate with AMPCol coating encouraged a continued antimicrobial activity and hence stopped the formation of biofilm [54]. Besides this, various soluble amyloid-β oligomers interact with membrane, soluble in intracellular space which is the most toxic form of the peptide, sometime may infest the brain for a longer time causing apparent damage in Alzheimer's disease [55, 56].

3. FUTURE SAFETY ASPECTS OF BIOLOGICALLY ACTIVE PEPTIDES

Furthermore, *in silico* techniques/methods can be employed to investigate or improve biologically

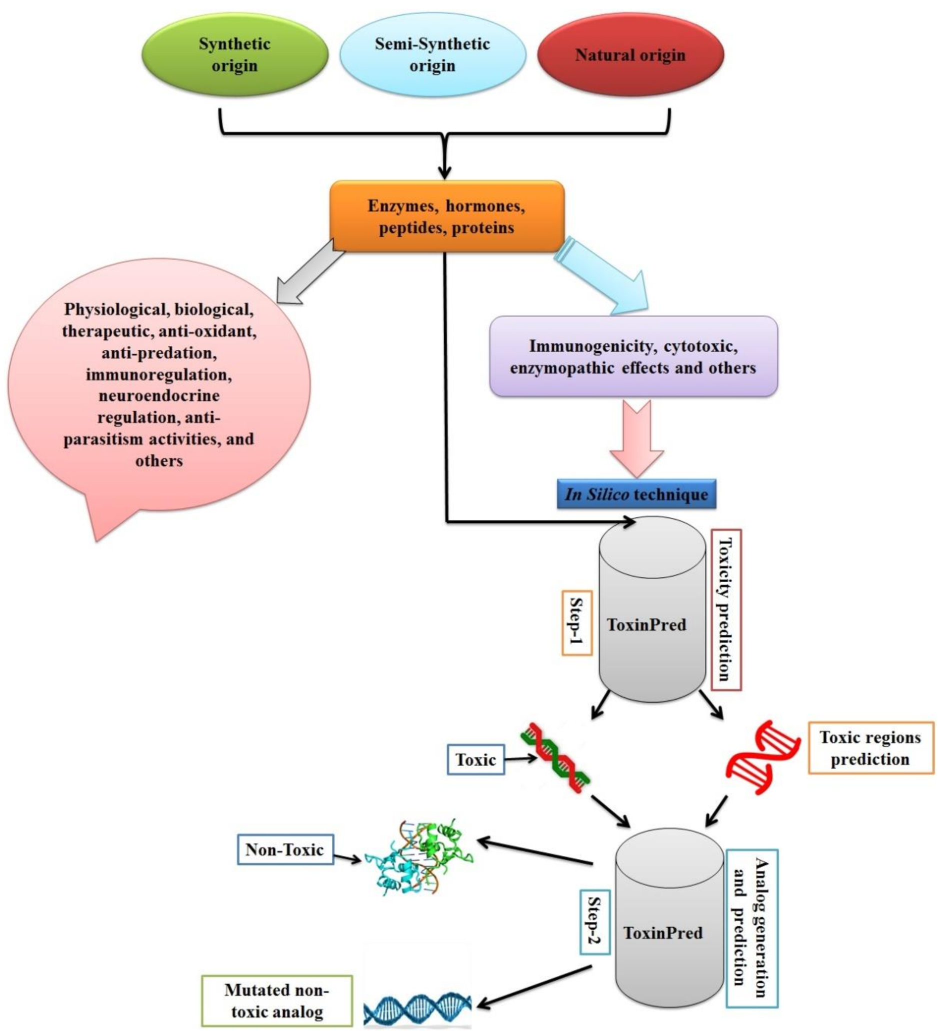

Fig. (1). Biologically active peptides and their possible toxicity prediction via *in silico* technique such as ToxinPred.

active compounds, which show a strong affinity for a particular target. The use of computational techniques is not only limited to the discovery of natural drugs, but it can also be applied for the identification and testing of the new bioactive peptides that possess a strong affinity for specific targets. In order to achieve the *in silico* approaches, it is important to design both *in vivo* and *in vitro* studies to evaluate peptide toxicity [57, 58, 59]. This will help verifying the possible toxicity of biologically active peptides. There are numerous identified compounds that have been screened through *in silico* method, which were not previously evaluated through *in vitro* and *in vivo* assays in order to get either harmful or beneficial response. The *in silico* methodological approaches are used to make different models and to test the toxicities of compounds, especially when there is a lack of data or the available data is insufficient. In the near future, biologically active peptides can be used for the diagnosis and treatment of cancer. At the moment, the introduction of of targeted chemotherapy and new drug delivery techniques is considered to be a useful tool to minimize the problems occurring during the conventional chemotherapy strategies. Along with already available peptides-based cancer treatment strategies such as cancer vaccines, tumor targeting by cytotoxic drugs and radioisotopes together with anti-angiogenic peptides are in clinical trials with the hope for improved efficacy. There are some examples of biologically active peptides which are in the late phase of clinical trials such as Stimuvax and Primovax, the first one is a palmitoylated peptide vaccine, which can be used for lung cancer while the second one is the peptide-based cancer vaccine [60, 61] as these are peptide in nature. Due to the remarkable advancements in the area of large-scale synthesis of biological peptides, it will be possible in the future to further develop drugs derived from peptides for the treatment of cancer, that will be available at an affordable price to the patients [62, 63]. There are certain technical obstacles in the development of effective peptide-based therapeutics which need to be addressed in the near future. As the majority of the peptide synthesis depends on expensive reagents such as resins and amino acids, therefore cheaper methods for the synthesis of peptides are needed to be introduced. This can be achieved by the application of molecular biology techniques such as a recombinant peptide expression.

In order to manufacture more peptide-based drugs, it is necessary to keep under consideration the already existing rational drug design techniques. By doing this, we can overcome the limitations that may occur in the formulation of such drugs. Also, the application of new emerging peptides such as multifunctional peptides, cell penetrating peptides and the peptide-drug conjugates, will add more to expand the therapeutic applications of the peptides especially through the use of *in silico* techniques [63-65].

Similarly, improving peptides screening and introducing computational biology to the field of peptides and peptide-based drugs will help new peptide drug discovery. The new emerging techniques such as metabolomics, proteomics and genomic screening of the toxins and other naturally available products can contribute to identifying biologically active peptides which may possess special structural features produced by uncommon post-translation modifications. Finally, the design of new peptides-based drug delivery systems, its formulation and extension in the half-life strategies will further help bring this new class of molecules to the market.

CONCLUSION

It is concluded that besides physiological role, peptides toxicity is associated with certain disorders such as celiac disease, cytotoxicity and immunogenicity. Therefore, it is essential to test their toxicity. However, the prediction of peptides toxicity is a costly process. Hence, to overcome these limitations, computational approaches are highly important as it will predict the toxicity of any peptide at very early stages through the use of computational methods. It is necessary to design future research projects that should focus on the safety and toxicity of the biologically active peptides along with pharmacokinetics and pharmacodynamics aspects. Moreover, the application of metabolomics and proteomics techniques can be employed in order to investigate the effects of these peptides on the genetic and epigenetics pattern, hence to highlight the nutritional value and beneficial health effects of these peptides.

The *in silico* technique can also be used to design a model in order to investigate the toxicity pathways, especially when there is a lack of essential experimental data. Furthermore, the application of these techniques can help to estimate various physiochemical properties of peptide molecules which are associated with environmental fate and support.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for study.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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