Tópicos Multidisciplinares em Ciências Biológicas 3

Edson da Silva
(Organizador)

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APRESENTAÇÃO

A coleção “Tópicos Multidisciplinares em Ciências Biológicas” é uma obra composta por estudos de diferentes áreas das ciências biológicas e da saúde. A obra foi ampliada e recebeu mais 47 capítulos distribuídos em três volumes. Os e-books foram organizados por trabalhos resultantes de pesquisas, ensaios teóricos e vivências dos autores.

As ciências biológicas englobam áreas do conhecimento relacionadas às ciências da vida e incluem a biologia, a saúde humana e a saúde animal. Nesta obra, apresento textos completos e atuais sobre estudos desenvolvidos durante a formação acadêmica ou na prática profissional. Os autores são filiados a diversos cursos de graduação e de pós-graduação em ciências biológicas, saúde, tecnologia e áreas afins.

Em seus 15 capítulos o volume 3 aborda, de forma categorizada, os trabalhos de pesquisas e revisões narrativas ou ensaios teóricos que transitam nos vários caminhos da atuação em ciências biológicas e áreas correlatas. Neste volume você encontra textos sobre biologia celular e molecular, microbiologia, meio ambiente e muito mais.

Espero que as experiências compartilhadas neste volume contribuam para o enriquecimento de novas práticas profissionais com olhares multidisciplinares para as ciências biológicas e suas áreas afins. Agradeço aos autores que tornaram essa edição possível e desejo uma ótima leitura a todos.

Edson da Silva

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CAPÍTULO 5

POTENTIAL PHARMACOLOGICAL APPLICATIONS OF LECTINS

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ABSTRACT: The word lectin is derived from the Latin *legere* which means “to choose” or “to select” and was introduced in biochemistry in the second half of the 20th century. Such molecules are characterized as glycoproteins that generally do not have an enzymatic role while have at least one non-catalytic domain – lectins are reversibly and specifically associated with simple and complex carbohydrates. Lectins differ from all other proteins through this type of connection with carbohydrates, promoting the ability to agglutinate cells through interaction with specific glycids having a non-immune origin. The present work aims to review the specialized literature in order to list the potential pharmacological applications derived from lectin-carbohydrate specificity in its most varied approaches.
KEYWORDS: Protein, Lectin, Biological activities.

POTENCIAIS APLICAÇÕES FARMACOLÓGICAS DAS LECTINAS

RESUMO: A palavra lectina é derivada do latim *legere* que significa “para escolher” ou “selecionar” e foi introduzida na bioquímica na segunda metade do século XX. Tais moléculas são caracterizadas como glicoproteínas que geralmente não possuem papel enzimático e apresentam ao menos um domínio não-catalítico – as lectinas se associam de forma reversível e específica aos carboidratos simples e complexos. As lectinas diferem de todas as outras proteínas através desse tipo de ligação com carboidratos promovendo a habilidade de aglutinar células por meio da interação com glicídeos específicos apresentando uma origem não-imune. O presente trabalho tem por objetivo revisar a literatura especializada de maneira a elencar as potenciais aplicações farmacológicas decorridas da especificidade lectina-carboidrato em suas mais variadas abordagens.
PALAVRAS-CHAVE: Proteína, Lectina, Atividades Biológicas.

1 I INTRODUCTION

Lectins are characterized as proteins and / or glycoproteins that do not have an enzymatic role - except for chimerolectins - and have at least one non-catalytic domain, lectins are reversibly and specifically associated with simple and complex carbohydrates (PEUMANS; VAN DAMME, 1995; SANTOS SILVA *et al.*, 2019). The presence of a region known as carbohydrate recognizing domain (CRD) - conserved in different types of lectins - allows lectins to interact with specific glycids segments (NI; TIZARD, 1996; VAN HOLLE *et al.*, 2017).

This interaction occurs through hydrogen bonds as well as Van der Walls forces between the glycidic segments (carbohydrates) and the amino acid residues present in the lectin’s CRD. (HAMID *et al.*, 2013; LIS; SHARON, 1998). Lectins differ from all other proteins because of its type of carbohydrate binding which promotes the ability to agglutinate cells through interaction with specific carbohydrates while maintaining nonimmune origin (DE MEJÍA; PRISECARU, 2005; GOLDSTEIN *et al.*, 1980).

The cell agglutination process occurs through the interaction of lectins with the glycoconjugatespresent in the cell membrane, thus lectins promote cross-links between adjacent cells, agglutinating them(PEUMANS; VAN DAMME, 1995). When the agglutination process occurs through the binding of lectins to carbohydrates on the surface of red blood cells,it can be observed macroscopically in a process named ashemagglutination (ALONSO *et al*., 2001). At the end of the 19th century, microbiologist Peter Hermann Stillmark obtained the first evidence about thenature ofproteins that agglutinateserythrocytes, called hemagglutinins(GHAZARIAN; IDONI; OPPENHEIMER, 2011; SHARON; LIS, 2004). In 1888, Stillmark detected the presence of lectins by assessing the toxicity of protein extracts from seeds(*Ricinus communis*), the first hemagglutinin depicted was called ricin (STILLMARK, 1888).

The hemagglutination phenomenon occurs dueto the presence of glycoconjugates present on the outer N-terminal region of the largest transmembrane sialoglycoprotein of erythrocytes, Glycophorin A (GPA). Representing 2 to 4% of the erythrocyte membrane proteins, GPA is biochemically constituted by sialic acid and terminal carbohydrates, because ofits high concentration in the erythrocyte membrane GPA alone isresponsible for 80% of the negative charge of these cells(AUFFRAY *et al*., 2001). The importance of the negatively charged surface in the case of erythrocytes is the lower occurrence of cell-cell interaction and, in turn, erythrocyte agglutination, events that would hinder blood circulation. Glycophorin A is part of the class of glycophorins that have three domains: anexternaldomainto the membrane presenting oligosaccharides in its structure, a domain in the membrane bilayer and another in the internal portion in the cell cytoplasm. GPA differs from the others because its external domain is susceptible to modifications by enzymes such as trypsin, ficin and papain(MURADOR; DEFFUNE, 2007).

Itwas possible to improve the hemagglutination assays with lectins, because with commercially available proteases, trypsin and papain, for example, it is possible to modify the erythrocyte cell membrane in different parameters due to the action of these enzymes on the “scenario” of the surface of the membrane, allowing the interaction of lectins with their respective carbohydrates to be facilitated. The increase in agglutination in blood treated with proteases occurs due to the decrease in the net negative charge on the surface of erythrocytes and also through the cleavage of peptides and glycoproteins which, without the action of enzymes, can hinder the access of lectins by steric impediment, hindering hemagglutination(MURADOR; DEFFUNE, 2007; SCHNEBLI; ROEDER, 1976).

The hemagglutination experiment is a semi-quantitative experiment carried out using a bottom “V” or “U” microplate in which the serial dilution of the sample is made in which a possible lectin or the already isolated lectin is sought in which one wishes to observe the hemagglutinating activity. The dilution is done using 0.9% saline and after application of the sample, the addition of enzyme-treated and/or non-enzyme-treated red cells is added at a concentration of2-4%. After 30 minutes at room temperature the results are interpreted by hemagglutination units (UH) which is the inverse of the last dilution in which

the hemagglutination phenomenon was observed(FERREIRA *et al.*, 2018; MOREIRA; PERRONE, 1977).

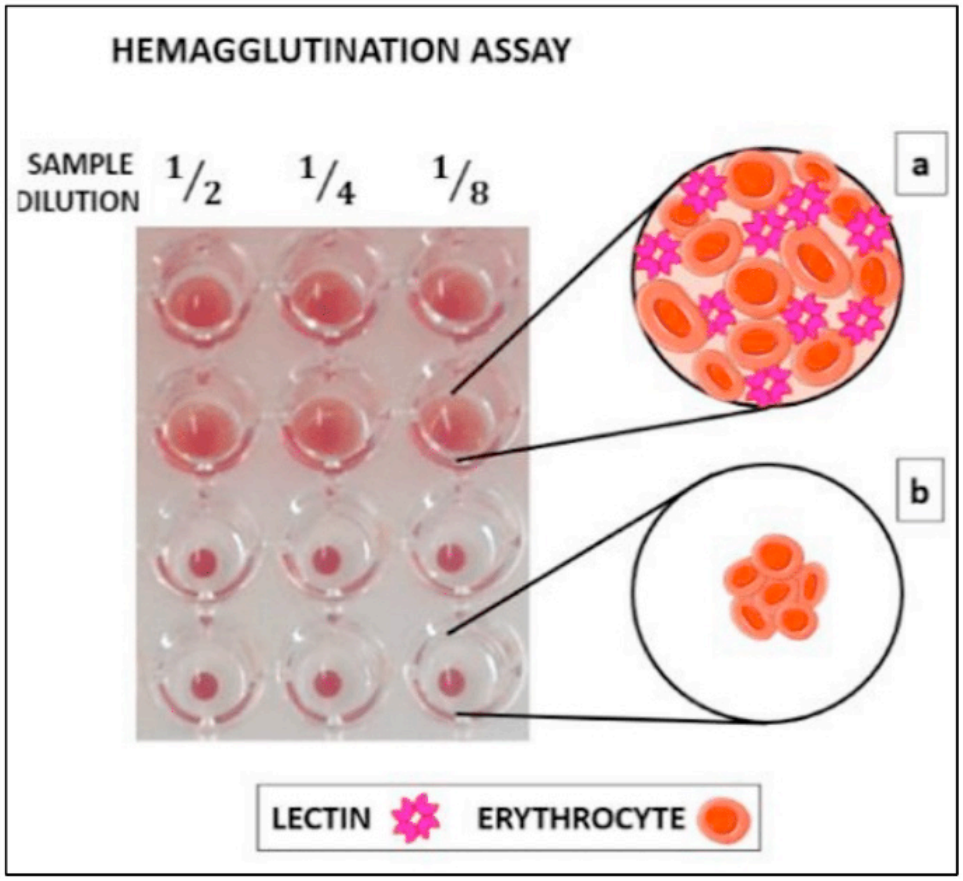

FIGURE 1. Hemagglutination test for the detection of lectins in "V" bottom microplates. (a) Formation of the erythrocyte network due to the presence of lectins; (b) Absence of hemagglutinating activity sedimentation of erythrocytes at the bottom of the well.

It can be observed an example with a real and fictitious image of how hemagglutination by lectins occurs in which the network of erythrocytes is formed(Figure 1), intertwined by the cross-linking of lectins with more than one CRD. This network of erythrocytes is possible to observe with the naked eye and allows the interpretation of results, when there is no presence of lectins in the tested samples, there is no formation of the erythrocyte network and these end up sinking at the bottom of the well, hence the importance of bottom microplates “V”or“U”. If we were to analyze the sample in figure 1 assuming that the hemagglutination was up to a 1/8 dilution, we will have a hemagglutin unit equal to 8 (UH = 8).

The modernization of the hemagglutination method to study lectin activity uses radioactive marking, increasing the sensitivity and precision of the experiment(HENDRICKSON; ZHERDEV, 2018). The use of the conventional hemagglutination experiment is widely explored in monitoring the purification steps and studying the most diverse sources, such as lectins, mainly from plants(BHUTIA *et al.*, 2019; DIAS*et al.*, 2015), sponges(GARDÈRES *et al.*, 2015), algae(SINGH; WALIA,

2018), fungi(KOBAYASHI; KAWAGISHI, 2016), bacteria(KUMAR; MITTAL, 2012)fish and crustaceans (JAYANTHI *et al*., 2017; LIU, XIAOBING *et al*., 2019), insects(VELAYUTHAM *et al*., 2017), snakes(KLEIN *et al*., 2015; NUNES *et al*., 2011), humans(MUKHERJEE *et al*., 2014).

Explaining their global structures related to their carbohydrate binding domain, according to Peumans & Van Damme (1995): Merolectins, lectins that have a single carbohydrate binding site, not being able to promote erythrocyte agglutination and neither precipitation glycoconjugates, an example of merolectins are those of the species Hevea brasiliensis, obtained from the latex of the rubber tree; Hololectins, are those that have at least two carbohydrate binding sites, agglutinate cells and precipitate glycoconjugates, encompassing a large part of the lectins from plants such as *Canavalia ensiformis*; Chimerolectins, have one or more binding sites with carbohydrates and another site with biological or catalytic activity, which can act -depending on their sites -as hololectins or merolectins. An example of chimerolectin is the species Ricinus communis, which inhibits ribosome activity; Superlectins, have at least two binding sites and each one can bind to different carbohydrates, an example is the lectin present in the bulb of *Tulipa gesnerianawhich* has a specific site for mannose and another for N-acetylgalactosamine.

2 | LECTINS AND ITS BIOLOGICAL ACTIVITIES

In addition to agglutination, the interaction between carbohydrates and lectins can play a key role in controlling normal and pathological processes in numerous organisms acting on many cell reactions such as cell growth, cell adhesion and cell division(FRANCISCO, 1991; ODINTSOVA *et al*., 2001; TANIDA *et al*., 2013). Just as this connection can act by inducing many different biological processes such as: characterization of ABO blood groups(MATSUI *et al*., 2001); bacterial agglutination (KHIN *et al*., 2000); lymphocytestransformation(KILPATRICK; GRAHAM; URBANIAK, 1986); inflammation (WALSH *et al*., 2005); apoptosisand protein regulation (PONRAJ *et al*., 2016); cytotoxicity(CHATTERJEE *et al*., 2016)tumorsand metastases(GORELIK; GALILI; RAZ, 2001); interaction between pathogensand itshosts(MEERT *et al*., 2014; SINGH; WALIA; KANWAR, 2016).

Recentstudies among countless of which report the participation of lectins in intracellular and extracellular processes that also have potential as therapeutic applications.(BISWAS, 2014).The biotechnological applications of lectins mainly involve their specific and high capacity to recognize carbohydrates, being targets as promising tools for biorecognition technology such as microarray techniques that use lectins to decipher biochemical “codes”that reflect the cell's physiological state(GEMEINER *et al*., 2009; YU; SHU; LI, 2020). Also used as isolation tools and in the mapping of cell surface glycoconjugates(XIE

et al., 2009). As well as affinity chromatography experiments with immobilization of lectins in the fixed matrix, as in the case of *Canavalia ensiformis* seed lectin that was efficient in separating glycoproteins from human serum(QIU; ZHANG; REGNIER, 2007).

Biorecognition through lectins can be used as a histochemical marker for the analysis of normal or altered human tissues(CAMPOS *et al*., 2006). Another example of a cell in which lectins can be used -through biorecognition, toxic action, inhibition and / or agglutination -is the intracellular parasite *Leishmania amazonenses* that has glycoconjugates on its cell surface with the function of recognition by the host cell as well as in the virulence of the parasite(ARANDA-SOUZA *et al*., 2018; JECNA *et al*., 2013).Lectins are also involved in differentiating between malignant and benign tumors and the degree of glycosylation associated with metastasis(LIU, BO; BIAN; BAO, 2010).

3 I ANTIBACTERIAL ACTIVITY OF LECTINS

The ability of lectins to interact with carbohydrates present on the surface of bacteria can prevent their mobility and growth, this interaction can change the structure of the bacteria and the permeability of its membrane(GAIDAMASH; STADEN, 2002). This characteristic can act in the identification of bacterial species through the difference of agglutination in microtiter plates, as in the experiment in which 23 lectin types were used and it was demonstrated that the lectin-bacterium interaction can be used as a possible tool to identify the species of Mycobacterium(ATHAMNA *et al*., 2006).

The lectin isolated from *Aplysia* sp. which is able to agglutinate *Escherichia coli* indicating its presence(GILBOA-GARBER; SUDAKEVITZ, 2001). The interaction of lectins and bacteria is based on the carbohydrates of cellular appearance and extracellular glycans, since bacteria expose carbohydrates on their surface, the carbohydrate-binding region plays a very important role for the interaction ofbacteria and lectins(SINGH; KAUR; SINGH, 2014).

Research works highlighting lectins as potential bacterial anti-biofilm tools are also found as: the antibiofilm activity of Bangladeshi potato lectin Pseudomonas aeruginosa(HASAN; OZEKI; KABIR, 2014); inhibition of the biofilm formation of *Staphylococcus aureus* and *Staphylococcus epidermidis* by the lectin purified from *Bothrops jararacussu* venom (KLEIN; FABRES-KLEIN; OLIVEIRA, 2015); and reduction of the biofilm formation of *S. aureus* and *Escherichia coli* by lectin from the marine sponge *Aplysina lactuca* (CARNEIRO et al., 2017).

Because they are proteins of non-immune origin capable of reversibly binding to carbohydrates, lectins can interact with the cell wall of bacteria and their extracellular glycans, often acting as an antibacterial molecule. (SINGH; BHARI; KAUR, 2010). Changes in cell permeability, due to the ability to form pores in the membrane, is the most explored

antibacterial mechanism of lectins (ARASU et al., 2017). Examples of this activity are found in the lectin of the mushroom *Sparassis latifolia* which was toxic against *E. coli*, a resistant strain of *S. aureus* and *P. aeruginosa* (CHANDRASEKARAN et al., 2016). The lectin found in the hemolymph of the crab *Portunus pelagicus* acted as an antibacterial agent against other species such as *Bacillus thuringiensis*, *Enterococcus faecalis, Proteus vulgaris, Citrobacter murliniae*, among others(JAYANTHI et al., 2017).

4 I LECTINS AND CANCER

In this last century, a large number of researchers have looked at the identification of new lectins, also studying their biomedical potential and their more intrinsic chemical characteristics, studies have sought to show that lectins, due to their potential to interact with cell membrane carbohydrates, could, also interfere in cellular communication networks, which are responsible for regulating various processes(PINHO; REIS, 2015).

Of all the human pathologies already described, cancer is one of the most difficult to decipher, since mutations that happen in a disordered way alter various aspects of cells, such as, for example, the glycosylation of several cellular proteins, being membrane proteins(WANG; AO; VUONG, 2011)and proteins secreted by cells, considered a priority in the study of these interactions(HAUTALA *et al.*, 2020).

Regarding the inhibition of cell proliferation, some lectins inhibit the proliferation of malignant cells through binding with the surface glycoconjugates, without necessarily being internalized (FERRIZ-MARTINEZ et al., 2010). The ability to prevent the proliferation of tumor cells was found in several lectins, such as in the lectin extracted from latex of the plant species *Euphorbia tirucalli*, which proved effective against HeLa (human adenocarcinoma) cell lines, PC3 (Prostate cancer), MDA-MB-231 and MCF-7 (breast cancer)(PALHARINI *et al.*, 2017), also in the lactose-binding lectin of the marine sponge *Cinachyrella apion*, which acted in the cell death of the human adenocarcinoma lineage (HeLa)(RABELO *et al.*, 2012)and lectin obtained in the fruiting bodies of the fungus *Clitocybe nebularis* (SABOTIČ; KOS, 2019)the latter was responsible for cytotoxic activity against leukemic T cells.

In addition to the interactions between lectins and cells that alter cell viability, lectins have recently been the subject of studies involving the detection of biomarkers, in the specific case of markers of the presence or stage of tumor cells(HASHIM; JAYAPALAN; LEE, 2017), this research is very relevant because many types of cancer have a very difficult early diagnosis, which can reduce the chances of patients controlling the disease at the right time and avoiding the process of metastasis(AL-AZRI, 2016).

5 I CONCLUSIONS

After all the approaches described above we can conclude that lectins have great potential in research both against some types of cancer, microbial infections and even biotechnological applications as shown in figure 2.

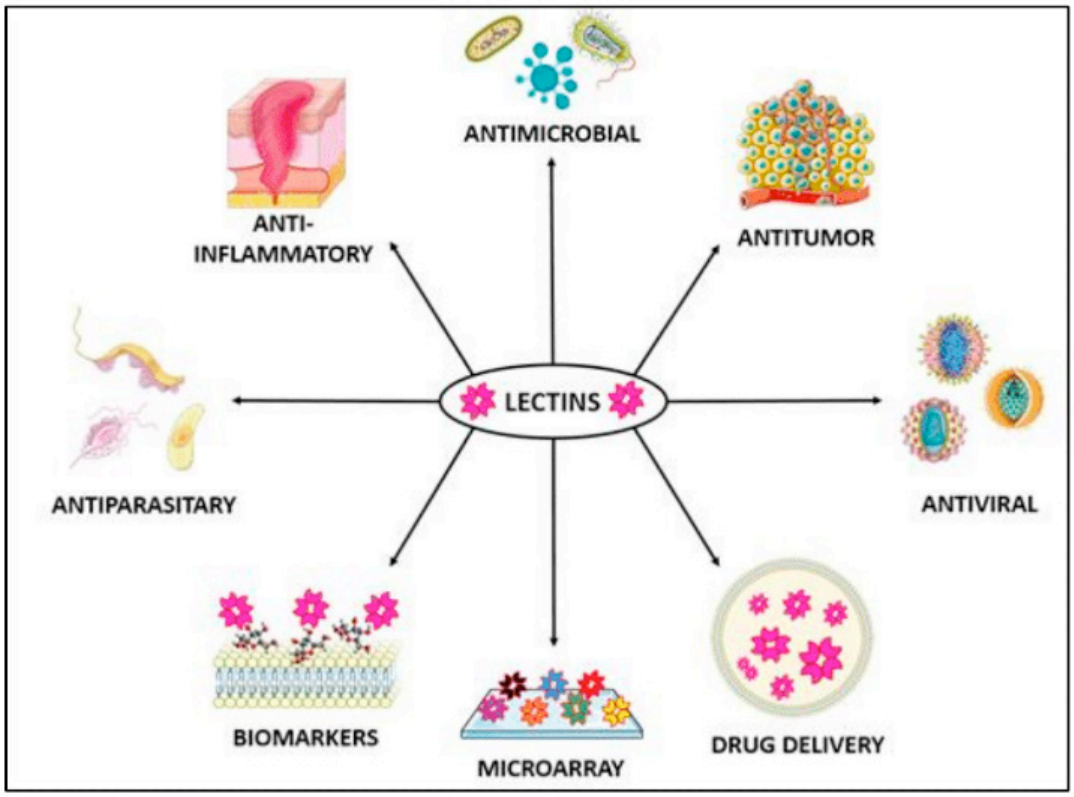

FIGURE 2. Lectins and their most diverse applications in science and biotechnology.

In addition to being widely distributed in the most different organisms, the analysis of its activity thanks to the hemagglutination technique that allows visualizing the action of its binding site and specificity, allowing researchers of this biomolecule to follow the stages of purification and later biological applicability of lectin. pure. Its application in treatments, diagnostics, identification and even addressing biomolecules has a long way to be explored in scientific research.

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