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Escola de Ciências Médicas e da Vida
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AVALIAÇÃO DO ESPECTRO DE ATIVIDADE CARDIOPROTETORA DA ALICINA

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Evaluation of the cardioprotective activity spectrum of allicin

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O alho (Allium sativum) possui ativos biológicos com propriedades cicatrizantes, com ação anti-inflamatória e cicatrizante, principalmente alicina (AC), dissulfeto de dialila (DADS) e aliina (ALL). Um dos compostos mais ativos é a alicina, que só existe depois que o alho é ferido e ativa a enzima aliinase que metaboliza a aliina em AC. Dentro deste contexto, abordagens *in silico* auxiliam na identificação mais rápida de alvos biológicos e potenciais ligantes promissores. O objetivo é avaliar o espectro de atividade biológica da alicina utilizando ferramentas *in silico*, abrindo perspectivas para futuros testes com esta molécula isolada. O método inclui levantamento da literatura, as estruturas identificadas foram codificadas no site PubChem e submetidas à análise preditiva de atividade biológica, alvos moleculares e farmacocinética, utilizando os servidores online do PASS. Verificou-se que a alicina é a molécula mais promissora para ter atividade cardioprotetora, pois atende aos critérios necessários para se tornar um medicamento oral. Além disso, possui alta absorção intestinal e alta capacidade de atravessar a barreira hematoencefálica, promovendo uma boa ação sistêmica para um potencial cardioprotetor.

Garlic (Allium sativum) has biological actives with healing properties, with anti-inflammatory and healing action, especially allicin (AC), diallyl disulfide (DADS) and alliin (ALL). One of the most active compounds is allicin, which only exists after the garlic is injured and activates the alliinase enzyme that metabolizes alliin into AC. Within this context, in silico approaches help in the faster identification of biological targets and promising potential ligands.The objective is to evaluate the spectrum of biological activity of allicin using in silico tools, opening perspectives for future tests with this isolated molecule.
The method includes surveying the literature, the identified structures were coded using the PubChem website and subjected to predictive analysis of biological activity, molecular targets and pharmacokinetics, using the online PASS servers. It was found Allicin is the most promising molecule to have cardioprotective activity because it meets the necessary criteria that one needs to have to become an oral drug. In addition, it has a high intestinal absorption and a high capacity to cross the blood-brain barrier, promoting a good systemic action for a cardioprotective potential.

Keywords: Cardiovascular diseases; *In silico* modelling; *Allium sativum.*

Palavras-chave: Doenças Cardiovasculares; Modelagem *In sílico*; Allium sativum.

1. INTRODUCTION

The medical use of plants has been occurring since ancient civilizations as a treatment for many diseases and conditions. In this context, the Chinese pharmacopeia “Shen Nung Pen Ts’ao Ching” written in the first century B.C describes the medical use of Cannabis. In addition, many plants were used in Japan, India, Europe, and Egypt to treat illnesses that affected society at the time and were even fatal, like diarrhea and constipation (1).

Therapeutic use of plants was made by using the whole plant or part of it, either alone or mixed with other plants, to create the remedy and to achieve the desired effect. As for the administration of the medicine, there was oral use, topic, and inhalation. With the development of society and new technologies, studying those mixtures allowed the discovery of the active principles of plants and the development of specific drugs (1,2).

Garlic is a particular plant that has been widely used as a condiment in many cultures. Its cultivation dates back to over 4000 years ago in Egypt and is present mainly in Turkey. Beyond its culinary uses, it was also used in embalming processes and as a medicine for illnesses of the heart and blood, as a painkiller, infections, rheumatism, high cholesterol, and diabetes. Furthermore, it has been described that Hippocrates prescribed garlic as a treatment for wounds and urinary affections (2).

As for the biologically active compounds of garlic that present healing properties, there is allicin (AC), diallyl disulfide (DADS), alliin, diallyl trisulfide (DATS), diallyl sulfide (DAS), ajoene and S-allylcysteine (SAC). One of the most active compounds is allicin, which only exists after the garlic is injured and activates the enzyme alliinase that metabolizes alliin into allicin (3).

The leading cause of death in the world is cardiovascular disease (CVD), with approximately 17.9 million lives lost annually. In Brazil, the CVD is responsible for 30% of all deaths yearly, with at least 400.000 deaths. CVD is developed with risk factors like age, genetic heritage, gender, poor nutrition, sedentary lifestyle, smoking, and type 2 diabetes (4,5).

That being said, this disease can be prevented, or at least its outcome changed by lifestyle modifications and proper medications. In Brazil, CVD is an economic and public health problem, with the disease's direct and indirect costs increasing each year, the direct cost estimated by the treatment, and the indirect by the morbidity (5,6).

In silico approaches are a mixed use of cheminformatics, virtual screening, target fishing, and molecular docking to find a biological target (BT) that can be used to treat a particular disease. Usually, the development of new drugs is made by discovering a new molecule that can act at the BT. However, this method is more expensive and challenging than the *in silico* approaches, making this last one more exciting for developing drugs (7).

Allicin is a natural compound of garlic that demonstrates some cardioprotective action, which can be a powerful tool in preventing CVD. However, a lot of structural diversity and complexity makes it harder to develop a drug that can act at a BT and be effective. Thankfully, developing *in silico* approaches can improve the number of BTs and help create more selective drugs (3,7).

The objective of this study was to evaluate the spectrum of biological activity of allicin from computational tools, opening perspectives for future tests with this isolated molecule.

2. METHODS

A virtual search was realized for the plant with a possible cardioprotective activity on PubMed platforms (https;//pubmed.ncbi.nlm.nih.gov/), ScienceDirect (https://scielo.org/), where articles about *Allium sativum* L. were selected.

Molecular structures were obtained from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). In the first screening of molecules, the PASS Prediction server (http://www.pharmaexpert.ru/passonline/) was used to structurally select the molecules with the central probability of having cardioprotective activity. The pharmacokinetic prediction analysis was performed using the SwissADME server (http://www.swissadme.ch/), and a toxicological prediction was fulfilled in Protox II (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6031011/). In the end, a

projection was performed to verify the most likely targets of the selected ligands using the Swiss Target Prediction server (http://www.swisstargetprediction.ch/).

3. RESULTS

After a literature review, we found that the medicinal properties linked to garlic are cardioprotective, antimicrobial, antiseptic, antifungal, antiviral, antioxidant, anticancer, immunostimulating, hypoglycemic are related to allicin, which was presented as its main bioactive compound. Using PASS prediction, from the name of our compound, the structural formula was found, and then the pharmacological effects, mechanisms of action, toxic and adverse effects, and the interaction with metabolic enzymes and transporters. Then, starting from the idea that components with similar structures have similar biological activities, predictions are established with the expectation of these same behaviors. For this, it carefully demonstrates a value Pa>0,7 that corresponds to the highest probability that the activity described by the software is, in fact, the real one.

The present study selected allicin after confirming that it was a substance with potential gastrointestinal absorption, without permeability to the blood-brain barrier, and lower interactions with cytochrome P450. This is because, according to Lipinski's criteria, a substance will only be recognized as a druglike from the moment it can meet five predetermined criteria. Are the: molecular weight is less than 500 Da, the calculated octanol/water partition coefficient (log P) is less than 5, the compound has no more than 5 hydrogen bond donors, and the molecule has no more than 10 hydrogen bond acceptors. These five criteria guarantee that this candidate molecule has excellent chances of being orally bioavailable. In addition, they also evaluated blood-brain barrier permeability and interaction with cytochromes at the hepatic level. To know the drugs, the similarities of components are sought, and consequently, the possible structural interactions made are predicted(Figure 1).

The next step to define and predict the toxicity of the studied compound was to use the Protox II platform, which established safe dosage parameters based on hepatotoxicity, carcinogenesis, and mutagenesis activity. Allicin (Figure 1) showed a median lethal dose (LD_{50}) of 874mg/kg and a predicted toxicity class 4(Table 1). The prediction accuracy was about 54,26%, and the average similarity

was 47,61%. However, the toxicity model report has evidenced hepatotoxicity, immunotoxicity, and carcinogenicity; allicin is still safe because they need high doses to generate toxicities. On a scale from 1 to 6, with 1 being the most toxic and 6 the least harmful, allicin obtained class 4.

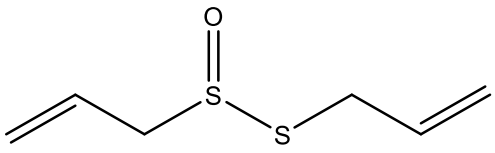

Figure 1. Molecular structure of allicin.

SwissTargetPrediction tool helps us to estimate the most probable macromolecular targets of a small molecule bioactive. The prediction algorithm has technical information that can say about the interactions landscape of bioactive molecules. In our search to interact with a biologically active target, we look for a similarity between the structures of target molecules and ligands known from humans(Table 2). Those cheminformatics analyses let us minimize animal uses, with less cost and time, by alternative research methods instead of traditional scientific research in vitro. Allicin was evaluated for interaction with human receptors and and its component that stands out the most as having cardioprotective activity was the 11-beta-hydroxysteroid dehydrogenase 1, that has the ability to controls the reversible conversion of biologically active; participates in the corticosteroid receptor-mediated anti-inflammatory response, as well as metabolic and homeostatic processes; bidirectional in vitro, predominantly functions as a reductase in vivo, thereby increasing the concentration of active glucocorticoids; it has broad substrate specificity, besides glucocorticoids, it accepts other steroid and sterol substrates, catalyzes the stereo-specific conversion of the significant dietary oxysterol; 7-ketocholesterol (7-oxocholesterol), into the more polar 7-beta-hydroxycholesterol metabolite, 7-oxocholesterol is one of the most essential oxysterols, it participates in several events such as induction of apoptosis, accumulation in atherosclerotic lesions, lipid peroxidation, and installation of foam cell formation; and catalyzes the synthesis of 7-beta-25-dihydroxycholesterol from 7-oxo-25-hydroxycholesterol in vitro, which acts as ligand for the G-protein-coupled receptor (GPCR) Epstein-

Barr virus-induced gene 2 (EBI2) and may thereby regulate immune cell migration.

Table-1 Pharmackinetics properties of allicin compound.

Druglikeness	Yes
Gastrointestinal absorption	High
Blood-brain barrier permeant	Yes
CYP450 inhbitor	None

Table-2 Target prediction of allicin compound.

Target	Common name	Target Class
11-beta-hydroxysteroid dehydrogenase 1	**HSD11B1**	**Enzyme**
Acyl coenzyme A:cholesterol acyltransferase	CES1	Enzyme
Carboxylesterase 2	CES2	Enzyme
Carbonic anhydrase XII	CA12	Lyase
Carbonic anhydrase IX	CA9	Lyase
Serotonin 6 (5-HT6) receptor	HTR6	Family A G protein-coupled receptor
Adenosine A1 receptor (by homology)	ADORA1	Family A G protein-coupled receptor
Metabotropic glutamate receptor 4	GRM4	Family C G protein-coupled receptor
Vascular endothelial growth factor receptor 2	**KDR**	**Kinase**
Serine/threonine-protein kinase Aurora-A	AURKA	Kinase
Dopamine transporter (by homology)	SLC6A3	Electrochemical transporter
Heme oxygenase 1 (by homology)	HMOX1	Enzyme
Arachidonate 15-lipoxygenase	**ALOX15**	**Enzyme**
Dual specificity phosphatase Cdc25A	CDC25A	Phosphatase
Poly [ADP-ribose] polymerase-1	PARP1	Enzyme

4. DISCUSSION

Many components of garlic and different preparations (oil, powder, liquid extract) have shown cardioprotective capacities. Therefore, there are many ways that these components and preparations can reduce cardiovascular risk. The first component that we are going to analyze is DADS. DADS has shown the ability to lower cholesterol, similarly to statins, by inhibiting 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase; meanwhile, SAC presented the ability to significantly reduce total cholesterol and triglycerides, although the mechanism is not fully understood(8).

Different studies found contradicting results when it comes to the effects of garlic on blood lipid parameters. Still, most studies found some level of lipid-lowering action, which is a very promising finding in the interest of reduction of cardiovascular events, such as heart attacks. One clinical trial showed that a garlic powder tablet of 400 mg could reduce total cholesterol, LDL-cholesterol, and triglycerides and increase HDL-cholesterol(8).

A few interesting findings were that the aged garlic extract (AGE) presented the ability to decrease coronary artery calcification (CAC), systolic blood pressure (SBP), and diastolic blood pressure (DBP). Not only AGE, but other garlic-based preparations have shown hypotensive effects. These effects are believed to be through inhibition of ACE; downregulation of angiotensin II receptor, stimulation of NO and H_2S; reduction of the synthesis of vasoconstrictor prostanoids. CAC is an excellent predictor of future cardiovascular events as also a high SBP, and although DBP is not the most critical risk factor related to CVD, it influences the development of the disease (9,10).

Another critical effect of AGE is its beneficial decrease in pro-inflammatory interleukin-6 (IL-6). This interleukin plays an essential role in atherosclerosis development, which can later contribute to the development of CAC. Although its not only through down-regulation of IL-6 that garlic contributes to the reduction of atherosclerosis, there is also the inhibition of carotid intima-media thickness observed in groups using garlic powder tablets. As a result, these garlic-based preparations can prevent future cardiovascular events by immunomodulation(9,11–14).

It is also believed that AGE can act as a cardioprotector by playing a role in the pathogenesis of the vascular disease through the decrease of vascular

oxidative stress, vascular endothelial function, and platelet function. Although it's not yet confirmed, many studies have suggested those actions(12,14).

5. CONCLUSION

Cardiovascular diseases remain at the top of the leading causes of death worldwide. Together with this increase, interest in the preventative effects of medicinal plants is getting bigger. The pharmacophore approach utilized here has demonstrated that allicin is bioactive molecule most promising of those present in garlic to develop cardioprotective mechanisms as it behaves similar to receptors that interfere in stimuli and reactions linked to cardiac activities. These studies can help to elucidate the relevant effects of Allicin and the results described herein may encourage and help focus studies of other plant species. This could be a starting point in developing novel therapeutic options against cardiovascular diseases, mainly using natural sources that have the potential to be more effective, more accessible and with fewer side effects than drugs already recognized. In addition, this study takes knowledge about garlic's properties to another level. It's also possible to observe the necessity of new types of drugs and the molecular mechanism in order to increase what is known about this specific field. At last, this article provides information to encourage in the conception of new in vitro and in vivo assays with this organosulfur compound.

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