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IN SILICO AND *IN VITRO* STUDY OF DPP-IV ENZYME ACTIVITY IN PRESENCE OF KD PEPTIDE

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SUMMARY

Type 2 Diabetes Mellitus (DM) contributes to increased morbidity and mortality from chronic non-communicable diseases in illnesses (NCDs) such as cholesterol and cardiovascular problems. In Brazil, cowpea is cultivated mainly the semi-arid sertão of the Northeast region, and in small areas in the Amazon. The syncretins GIP and GLP-1 are part of the glucagon-like peptide family, so there is some correlation in the amino acid sequence between these peptides. These peptides bind to six specific GIP and GLP-1 receptors and are rapidly metabolized by the enzyme dipeptidyl peptidase-IV (DPP-IV). The objective of this study is to compare, using various *in silico* tools , the binding of the DPP-IV enzyme to peptide KD and also the bioactivity through the Biopep sites. Through *in-silico* and *in* vitro analyses, the KD peptide is expected to bind to the DPP-IV enzyme of the bean protein, causing glycemia inhibition in patients with type 2 diabetes. Therefore, the analyses are important for generating easily accessible and cost-effective exploratory results. Results and Discussion, Conclusion.

Keywords: Type 2 Diabetes Mellitus. Cowpea. Dipeptidyl peptidase-IV DPP-IV.

ABSTRACT

. Type 2 Diabetes Mellitus (DM) contributes to increased morbidity and mortality from chronic non-communicable diseases (NCDs) such as cholesterol and cardiovascular problems. In Brazil, cowpea is cultivated mainly in the semi-arid sertão of the Northeast region, and in small areas in the Amazon. The syncretins GIP and GLP-1 are part of the glucagon-like peptide family, so there is some correlation in the amino acid sequence between these peptides. These peptides bind to six specific GIP and GLP-1 receptors and are rapidly metabolized by the enzyme dipeptidyl peptidase-IV (DPP-IV). The objective of this study is to compare, using various in silico tools, the binding of the DPP-IV enzyme to peptide KD and also the bioactivity through the Biopep sites. Through in-silico and in vitro analyses, the KD peptide is expected to bind to the DPP-IV enzyme of the bean protein, causing glycemia inhibition in patients with type 2 diabetes. Therefore, the analyzes are important for generating easily accessible and cost-effective exploratory results. Results and Discussion, Conclusion.

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1 INTRODUCTION

Type 2 Diabetes Mellitus (DM) contributes to increased morbidity and mortality.
mortality from chronic non-communicable diseases (NCDs) such as cholesterol, and problems
cardiovascular diseases, among others. The relationship between DM and cardiovascular diseases leads to
possibility of the collective, that is, the two are associated with the same genetic type and the same
environmental background, with insulin resistance classified as one of the main
possible antecedents (CERIELLO; MOTZ, 2004).

In Brazil, cowpea is grown mainly in the semi-arid hinterland of the Northeast region.
and in small areas in the Amazon (FROTA; MAIA, 1996). In this case in the Northeast, the
production and productivity are 429,375 tons and 303.5 kg.ha–1, in that order. The
the largest producers are the states of Ceará, Piauí, Bahia and Maranhão, as well as the
largest planted areas (IBGE, 2008). The productivity of grains, dry or green, is
reserved primarily for consumption by the population to maintain a diet
nutritious, in preserved or dehydrated form (EMBRAPA, 2003).

Thus, the justification for this final course work is to study and analyze the
potential of DPP-IV enzyme inhibition activity in the presence of bean peptide
cowpea.

Aminopeptidyl peptidase (DPP-IV) alters the activity of several regulatory peptides
by selective removal of two amino acids from the amino terminus. The insulinotropic activity
Circulating GLP-1 is rapidly lost (t1/2 1-2 min) by cleavage by this enzyme
(ALVES; MEDINA; NEVES; 2007)

The enzyme dipeptidyl peptidase IV (DPP-IV) has become a major target for
treatment of DM2, this enzyme is responsible for the degradation of *GLP-1 ,* Peptide 1
similar to glucagon. Thus, GLP-1 occurred due to its effect on the ÿ cell of the pancreas. With
Therefore, syncretins are produced by the gastrointestinal tract and act by increasing insulin secretion
when eating food, but its action is inhibited in a short time by the action of enzymes such as
the DPP-IV (FERREIRA VA; CAMPOS, 2014).

The 2 main incretin hormones are polypeptides that inhibit gastric
GIP, Gastric Inhibitory Polypeptide and GLP-1, this peptide is similar to glucagon
so that there is some correlation of the sequence of the structure of the essential amino acids,
among the peptides, glucagon, in addition to being between GIP and GLP-1. 5,6 GIP is a peptide of
42 amino acids, cleaved from its precursor peptide, ProGIP, in contrast GLP-1 is cleaved

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of the pro-glucagon precursor and includes peptides of 30 and 31 amino acids. GIP and GLP-1 are secreted by the gastrointestinal tract. Thus, GIP is absorbed by the K cells of the our body, located especially in the duodenum and proximal jejunum. GLP-1 is secreted by L cells, found primarily in the ileum and colon. After release from incretins soon after oral consumption of nutrients, such as carbohydrates, lipids and proteins, that most stimulates the secretion of the GIP hormone are carbohydrates, specifically (CHACRA; ARODA; HENRY, 2006). Thus, when these peptides bind to its six specific GIP and GLP-1 receptors, has the ability to carry out metabolism of enzyme dipeptidyl peptidase-IV (DPP-IV) (MOLLER, 2001).

Thus, the question is: What is the effect of the KD peptide on the DPP-4 enzyme and its consequent action as a drug treatment for patients with type 2 diabetes.

The initial hypothesis points to the binding of the DPP-4 enzyme to the KD peptide, acting as well as treatment and prevention of type 2 diabetes.

Thus, the general objective of this study is to determine the inhibition capacity of DPP-IV enzyme by the KD peptide and its possible bioactivity.

Recent studies indicate that dietary protein does not only have a building function and energetic, but also plays an important role in the bioactivity of precursors of biologically active peptides in which they play a functional physiological role (CORREA IN; POLTRONIERI F; MARQUES 2016).

2 THEORETICAL FRAMEWORK

2.1 THE COWPEA BEAN

Cowpea can restore the balance of some minerals such as iron and zinc. (CRUZ, 2000), especially because these micronutrients point to primordial functions for being a constituent of blood and enzymes involved in electron transfer, in addition to synthesis of protein and nucleic acids, carbohydrate metabolism, among others (CAIRO et al., 2002;RIOS, K.KING, SHAMES; WOODHOUSE, 2000).

Biochemical studies of domesticated plant seeds and the continued production of new cultivars obtained through genetic improvement techniques have demonstrated important changes in the chemical composition of seeds and even in the expression of proteins

involved in plant defense (CHRISPEELS, RAIKHEL, 1991), making them suitable for human consumption, with flavor and nutritional conditions for a healthy diet.

Cowpea grown in Brazil, mainly in the semi-arid backlands of the region Northeast and in small areas of the Amazon. The largest producers are highlighted States of Ceará, Piauí, Bahia and Maranhão. To which production of this legume is destined for human consumption in its natural form, as well as in preserved or dehydrated. With this, through the centesimal analysis of cowpea flour, in which they are form of raw material is an excellent protein source, for which it obtained 36.47% of its centesimal composition (CORREA IN; POLTRONIERI F; MARQUES 2016).

According to Salgado *et al.* (2005), it was identified that in ready-to-eat cowpea, has a quantity of 29.3% of total carbohydrates. As for the qualitative aspect, it was found that 9.1% of total dietary fiber, (SALGADO *et* al., 2005).

In the BRS-Milênio cultivar, a percentage of 27.4% of the total carbohydrates was found referring to the total dietary fiber content, and 14.2% of which 14% are fiber are represented by soluble ones. Therefore, the results demonstrate that cowpea has a high content of dietary fiber, in addition to increasing water absorption in the colon, preventing constipation, and essential in the prevention and promotion of health, and can contribute to reducing the risk of chronic non-communicable diseases such as cancer, cardiovascular disease and diabetes mellitus (FROTA KM G *et al,* 2009; FAIVRE, BONITHON-KOPP, 1999; FELDHEIM; WISKER, 2000).

Soil conditions and treatments can increase cowpea productivity, especially when observing the climate, irrigation management, cultural treatments, fertilization and residual effect of fertilizer , in which they observed a 25% increase in pod productivity and 50% of grain productivity, 100% above the indicated nitrogen doses were used, phosphorus and potassium. It was observed that the productivity of pods was influenced by judicious irrigation, achieving maximum technical efficiency for productivity of 8395.5 kg ha-1 with the blade 87% of the reference evapotranspiration (ROCHA; *et al* 2019).

With a short life cycle, the bean species studied may present late cultivars and early, low water requirements, hardiness and through symbiosis with bacteria genus Rhizobium fix nitrogen from the air. Their ease of vegetative development allows it to be cultivated in regions with rainfall rates between 250 and 500 mm and temperatures of 18 to 34°C in its cycle. Phenology has the function of following the periodic changes in the appearance and constitution of plants in the environment, under certain conditions conditions offered (PINHEIRO *et al,* 2019)

2.2. BIOACTIVE PEPTIDES

In the quest to improve the quality of human life, several studies on proteins of plant origin, found in legumes such as lupin, soybean, lentil and cowpea, with a great possibility of reducing risks, in the control or prevention of cardiovascular diseases, producing its hypocholesterolemic effect (BARBANA C, BOUCHER AC, BOYE JI 2011; DURANTI M, 2006; FLEET KMG, SOARES, RAM, ARÊAS JÁ, 2008; FONTANARI G *et al* 2012).

Studies have identified that the beneficial effects are related to enzymes bioactive peptides, resulting from the hydrolysis of these proteins, having a great importance in regulation and metabolic modulation of cholesterol. Peptides offer these benefits when they relate to the modulation, inhibition or regulation of some genes transporters or enzymes related to the inhibition of endogenous synthesis and intestinal absorption cholesterol (MACARULLA, et *al.,* 2001; MARCHESI, et *al.,* 2008).

Bioactive Peptides are capable of being developed from precursor protein by digestive enzymes during ingestion, processing, maturation, fermentation, cooking, storage or in vitro hydrolysis by proteolytic enzymes. As bioactive peptides are small sequences of food proteins, composed mainly of 2-20 residues of amino acids, which has positive physiological effects on human health. bioactive peptides contribute to a variety of targets, among which the following stand out: immune, cardiovascular, digestive and endocrine systems in the human body (SILVA, 2020).

Legumes are produced and consumed by humans, in many countries there are thousands years, but the discovery of the beneficial qualities of enzymes was only identified in studies from – 20 to 30 years ago – this interest in academic and research studies as a food with functional effects dates back 20 years. It has now been recognized that dietary protein not only has a great building function but also an energetic one, should produce a bioactive structure and/or with the possibility of being the future, for the development of biologically active peptides, which play a role in the structure functional physiological (DURANTI, 2001), considering that the main idea is to know whether a peptide has the effect of improving the quality of life of humanity, both in terms of food, energy and mainly in preventing chronic diseases.

In relation to nutritional value, the centesimal composition, expressed as a percentage of dry weight, showed that cowpea seeds have, on average, 22.01% protein, 60.57% carbohydrates, 2.75% lipids, 2.07% ash and 12.41% moisture. Showed

also that cowpea seeds have the eight essential amino acids, Threonine (1,24 mg/kg), Valine (1.22 mg/kg), Leucine (1.42 mg/kg), Isoleucine (1.37 mg/kg), Methionine (1.15 mg/kg), Phenylalanine (1.75 mg/kg), Lysine (1.58 mg/kg), Tryptophan (0.17 mg/kg) (ARAÚJO, COSTA, 1997).

During the studies it was observed how proteins and peptides present wide chemical and functional diversity. However, they are small molecules, presenting two to dozens of amino acid residues. The active peptides were discovered and had their chemical structures determined from the 1950s onwards. Since then, with the knowledge on these molecules, techniques were developed for their isolation, analysis, purification, identification and quantification, which were improved. With this, the need to synthesize these molecules and analogues of these, on different scales, allowing conducting studies on the biological role of natural peptides. Together, we also techniques were being developed for the manipulation, synthesis and cloning of genes that express these proteins/peptides (MACHADO *et* al., 2004).

Several strategies have been applied to improve production and reduction of peptide metabolism, along with the routes of administration, leading to increased commercialization of these drugs. Therapeutic peptides can be obtained from from different sources, such as extraction from natural sources; synthesis in animals or plants transgenic; solution or classical synthesis; solid phase synthesis; enzymatic synthesis; synthesis by recombinant DNA technology (SOUZA, 2017).

2.3. DPP-IV METABOLISM AND ITS ROLE IN TYPE 2 DIABETES

Diabetes is being considered the epidemic of our century, since in 2010 around 285 million individuals worldwide had the disease and the estimate is that by 2030, the number of people with diabetes will reach 435 million. Given the relationship to reduced glucose tolerance characteristic of pre-diabetic conditions the numbers in 2010 reached 344 million, with the number of diagnosed cases expected to reach 472 million One of the biological targets involved in the study of diabetes is the enzyme dipeptidyl peptidase-4 (DPP-4), DPP-4 inhibitors should decrease or potentiate the effects of peptides used in various pathologies such as systemic lupus erythematosus, rheumatoid arthritis, depression, schizophrenia, anorexia nervosa, fibromyalgia, immunodeficiency syndrome acquired (AIDS) and even graft rejection depending on the context of the disorder and its phase of evolution, as this enzyme has several substrates located in different places

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biological, acting in the regulation of the metabolism of peptide hormones, also in the immunological response of the human organism (PANTALEÃO, 2014).

During the development of the studies, a theoretical basis for Inhibitors of DPP-4 in the control of type 2 diabetes, when it was observed that incretins produced actions beneficial physiological changes during glycemic control activity, with great potential in type 2 treatment (NAUCK *et* al., 2004), as it is a chronic disease that exists in the world. It has been identified that syncretins are hormones synthesized in the gastrointestinal tract and secreted upon the entry of nutrients into the intestine (DOYLE, EGAN 2007; JANG *et* al.,2007), having an important role in blood glucose homeostasis by stimulating insulin secretion in a glucose-dependent manner (DRUCKER, NAUCK 2006, ;VILSBØLL, HOLST, 2004;). The predominant incretin hormone is the peptide-like 1 to glucagon (GLP-1), and studies have shown that GLP-1 was able to improve homeostasis of glucose, including the enhancement of insulin secretion in a glucose-dependent manner and inhibition of glucagon secretion (DOYLE; EGAN, 2007).

The first incretin hormone GIP was described, it is a single peptide with 42 amino acids. It circulates at low levels in the blood, but in response to glucose intake or fat, levels increase considerably, stimulating the production of endogenous insulin. At the same time, GIP also influences fat metabolism in adipocytes, stimulating lipoprotein lipase activity, stimulating beta cell proliferation Sitagliptin being the first DPP-4 inhibitor used in clinical practice, licensed in 2006, is quite selective for the DPP-4 enzyme, reaching a stable plasma concentration after three days of application. Maximum plasma levels are reached between one and two hours after taking the tablet, with a half-life of 8 to 14 hours, its bioavailability is 85%8 (FERREIRA VA; CAMPOS, 2014).

According to this information, it was planned to use GLP-1, initially as therapeutic measure in type 2 diabetes, during the process, studies reported that the secretion of this hormone in the state of diabetes is absent or even compromised, favoring deficiency in insulin secretion (NAUCK *et al.,* 2004). In later studies and research then admitted that the therapeutic use of GLP-1 as an antidiabetic agent was unfeasible due to its half-life is relatively short as a result of its rapid inactivation by the enzyme Dipeptidyl Peptidase-4 (DPP-4) (DRUCKER D. J, NAUCK MA, 2006; DEACON *et* al., 2008). GLP-1 is rapidly metabolized by DPP-4, with an active half-life of only 1 to 2 minutes (ANDUKURI *et al.,* 2009), making it difficult to use in the treatment of type 2. Previously, it had been demonstrated in studies that DPP-4 is also found in

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endothelium of capillaries draining the intestinal mucosa, where GLP-1-secreting cells are situated (GAUTIER *et al.,* 2008), which indicates that most of the GLP-1 is inactivated almost immediately after its secretion (LOTFY; SINGH;KALÁSZet al., 2011).

Known as CD26, dipeptidyl peptidase 4 (DPP4) is a glycoprotein transmembrane that is almost entirely present on the surface of many cells, including epithelial and endothelial cells of many tissues and in cells of the immune system. Playing an important role in the regulation of the immune system, DPP4, promoting the activation and proliferation of T cells, regulating the function of other cells immunological and stimulating the production of proinflammatory cytokines. DPP4 is present in the circulation in a soluble form, where it maintains its enzymatic activity. DPP4 degrades the glucagon-like peptide-1 (GLP-1) physiologically and plays a role in glucose metabolism. possessing many other substrates, including cytokines, chemokines and growth factors. Acting as a ligand for binding proteins and growth factors extracellular, DPP4 is a serine protease widely distributed in human tissues and functions as a multifunctional protein. From the lower respiratory tract, kidneys, liver, small intestine and prostate, DPP4, is present in the placenta, lung fibroblasts and lesions in the skin, muscles and central nervous system (Mota *et al,* 2022).

Being from a group of enzymes existing in the circulation and on the surface of multiple tissues, such as the liver, pancreas, spleen, kidneys, vascular endothelium, etc. (GREEN *et al.,* 2006), its activity consists of cleaving peptide chains in which proline or alanine is present as the second amino acid from the N-terminal end, including cytokines, growth factors growth, neuropeptides and incretin hormones (POSPISILIK *et* al., 2002).

Its structure comprises 4 domains: a short cytoplasmic domain (1-6), a transmembrane domain (TMD) (7-28), a flexible rod segment (29-39) and a domain extracellular (40-766), which can be further divided into a highly glycosylated region, a cysteine-rich region and the catalytic region (ROHRBORN *et* al., 2015), incretins are released by the intestine after food intake and exert a series of physiological effects in the pancreas that could culminate in the release of insulin for glycemic homeostasis, if they were not rapidly degraded by the action of DPP-4. Inhibitors of this enzyme extend the half-life of incretins, thus prolonging their physiological effects on cells pancreatic alpha and beta (Figure 1)

Figure 1 Physiological actions of DPP-4 inhibitors

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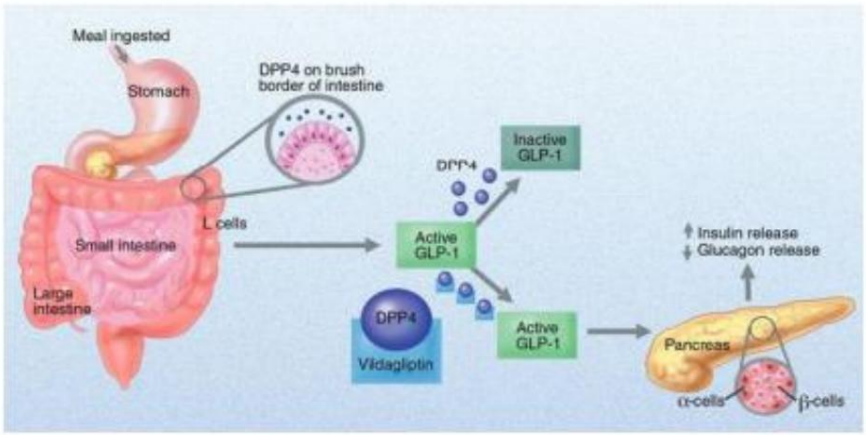


Source: Banerjee *et al.,* 2009

Dipeptidyl peptidase IV (DPPIV) is a product of the prolyloligopeptidase set of serine proteases. DPPIV removes dipeptides from the N-terminus of substrates, including many chemokines, neuropeptides, and peptide hormones. Specific inhibition of DPPIV is being investigated in human trials for the treatment of type 2 diabetes. the determinants molecular mechanisms underlying enzyme catalysis and substrate specificity, reports the structures crystalline DPPIV in free form and in complex with the first 10 residues of the substrate physiological, Neuropeptide. It has a free-form crystal structure of the enzyme reveals two potential channels through which the indicants can access the active site - the so-called propeller opening and side opening (AERTGEERTS et al, 2004).

2.2.1 ANALYSIS *IN SILICO*

With the development of the human genome it was possible to discover a great amount of data contained in ribonucleic acid (RNA), being of great importance the its constant storage and studies. With human development in control of technologies and academic studies directly related to molecular biology and genome was necessary to create a field of science, bioinformatics. Used in research for preventive and therapeutic diagnostic purposes, characterizing the biomedical application (PROSDOCIMI *F, et al.,* 2002).

Using computational experimentation, called in silico analysis, makes it possible build and simulate systems aimed at predicting properties and behaviors biological. To function properly, a cell depends on all its components participate in intracellular biochemical reactions and interact with each other. The cellular metabolism can be reproduced as a network formed by different types of

interactions, **such as :** gene-gene, protein-protein, gene-protein, gene-interaction metabolite and so on. The construction of a network is done from complex models mathematical and computational techniques that identify and characterize the dependence relationships of each gene (OLIVEIRA, 2014).

During bioinformatics research, identified as *in silico analysis,* it facilitates simulate chemical systems and construction of enzymes, to identify properties and biological behaviors (PALSSON B, 2000). The production of in silico approaches facilitates the time and expense required to bring a drug to market. Demonstrating the predictable absorption, distribution, metabolism, excretion and toxicity (ADMET) profiles, resulting in a rapid drug discovery process. These parameters pharmacokinetics are and evaluated by Admet-SAR (Dias, 2014).

Our human system needs healthy cells to function properly, as depend on all their components participating in intracellular biochemical reactions and interact with each other to produce healthy enzymes, which form the links of metabolism cellular, which can also be reproduced as a network formed by different types of interactions, such as: gene-gene, protein-protein, gene-protein, gene-metabolite and so on. Production of a network is done from complex models mathematicians and using bioinformatics to identify and characterize the relationships of dependence of each gene and/or enzyme contained in the cell. (RYBARCZYK-FILHO JL, 2011).

The *in silico* study is a method applied through data analysis in a a given species or genus, an aid to diagnosis through molecular analysis several computerized databases and programs that evaluate toxicity by determination of relationship structure-activity, put example the QuantitationStructureActivityRelationship (QSAR), the process by which the protocol relates the physical-chemical structure of a substance and its toxicity. These study processes have as their main objective to obtain fast, cheap, easy-to-implement methodologies and reproducibility that can be standardized and validated, an essential situation so that in vitro methods achieve international scientific acceptance (BAGLEY, et al).

With the use from the technique from HQSAR (of the English, HologramQuantitativeStructureActivityRelationships) producing a parameter that correlates the molecular hologram (fragments derived from the 2D structure and their respective contributions) with biological activity, based on the association of the distinction of fragments. In order to generate molecular fragments, some parameters are varied throughout throughout the analyses, such as distinguishing fragments using information about the

descriptors: atoms (A); bonds (B); connections (C); hydrogen atoms (H); chirality (Ch) and/or hydrogen bond acceptor and donor groups (DA); and also the size of the fragment (defined in number of atoms) and the length of the hologram (defined in bins, in quantity of binary descriptors referring to the fragment count).The molecular holograms are mathematical representations of the fragments of each compound and are related to the biological response through the use of multivariate data analysis techniques (PANTALEÃO, 2014).

Peptides have numerous advantages over small molecules that include high biological activity, high specificity, low production cost and high penetration. However, toxicity, immunogenicity and stability remain the main concerns in the development of hydroxypropyl methylcellulose-based drugs. peptides. Peptide stability can be increased in several ways, including the incorporation of D-amino acids (making peptides protease resistant), altering backbone chemistry, cyclization and incorporation of ÿ-aminooxyamino acids. Similarly, there are numerous *in silico* tools , which can predict the immunogenicity of peptides but there is hardly a way/method to predict the toxicity of peptides. Computational methods for predicting peptide toxicity not only save time and money, but also facilitate the design of better therapeutic peptides with low toxicity while maintaining the functionalities. *In silico* studies have the advantage of speed of execution, cost low operational costs and mainly does not require animals for toxicity testing, for the safety of its use in humans.

2.2.2 *IN VITRO* ANALYSIS

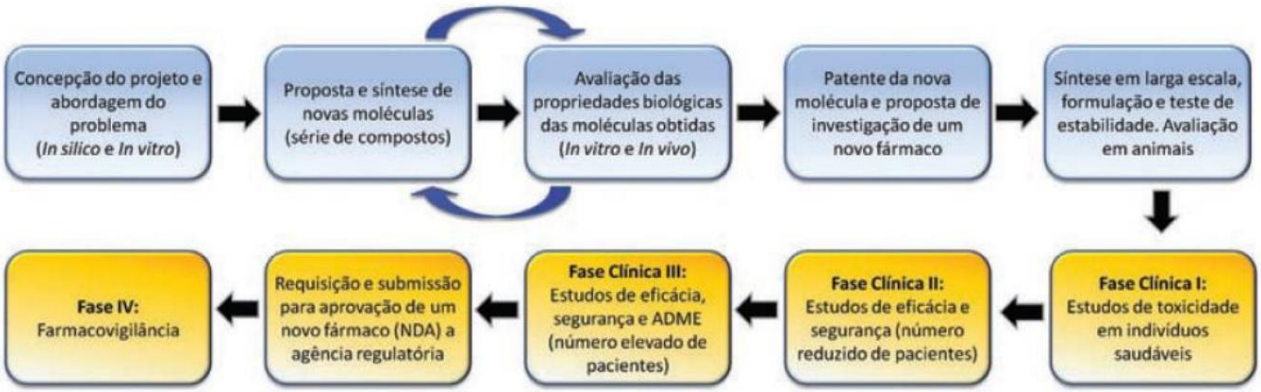
In vitro diagnostics is one of the major processes used by doctors and researchers as it is a more common procedure in academic and laboratory studies especially in the area of human health, it is not necessary to use *"in vivo"* which would be necessary to perform procedures on people or animals, one of the first methodologies described for *in vitro* evaluation , the biocompatibility test was suggested for to evaluate plastics used in medical-hospital articles. In the test, the materials were placed directly on a monolayer of mammalian cells and after 24 hours these cells were observed, as to the presence or absence of any toxic effect (MOSMANN, 1983).

The main purpose was to find a protocol that would replace the *in-house* tests. *in vivo* several other models have been developed, among them the so-called organotypic ones, the which use isolated organs from the animal, kept for a short period of time in vitro preserving its physiological and biochemical functions. With a focus on finding a protocol that replace in vivo tests, several other models were developed, including called organotypic, which employ isolated organs of the animal, kept for short periods

period of time in vitro preserving its physiological and biochemical functions. The importance validation, pre-validation, the three Rs concept (3 Rs), reduction, refinement and replacement, in the regulation of in vitro toxicological tests. Validation should be seen as a essential step in the development of the test and its acceptance should not be seen as obstacle, but rather a major process to accelerate the use of *in vitro* tests in the evaluation of risk of products for human use or that may cause damage to the environment (CRUZ *et al,* 2004).

Validation of the selected molecular target is essential for a number of reasons that involve everything from establishing their relevance in the pathophysiological process under study to the characterization of the impact of its selective modulation on the treatment or cure of diseases or dysfunctions in humans. Bioactive molecules (or ligands, from English *hits)* can be identified from real screenings (e.g., biological, biochemical) or virtual (e.g., example, computational) of natural products, synthetic compounds or collections combinatorial, and also through rational planning. Remembering however that, in all cases, biological properties must be determined experimentally, being necessary to develop high-quality standardized and validated tests, the figure 2 demonstrates the steps involved in the drug discovery and development process (ADME – absorption, distribution, metabolism and excretion; NDA – application for a new drug, from the English new drug application (GUIDO; ANDRICOPULO ; OLIVA, 2010).

Figure 2: Steps involved in the drug discovery and development process (ADME – absorption, distribution, metabolism and excretion; NDA – new drug application).



Source: (GUIDO; ANDRICOPULO; OLIVA,2010)

3 MATERIALS AND METHODS

The universe of Science is understood as a set of substances that deal with survey, established by the organization of scientific knowledge. This may involve from internal issues, such as scientific method and relationship between experimental procedure and theory, even external ones, such as the influence of social, cultural, religious and politicians in the acceptance or rejection of scientific ideas (MOURA, 2014).

According to authors Lakatose Marconi (2003), research is understood as "to investigate something thoroughly, is to investigate." The authors report that the meaning of term investigation "is not homogeneous, as there are many definitions of the term in different fields of understanding. However, the starting point of the research lies in the problem that a solution must be defined, evaluated, analyzed and then a solution attempted" (LAKATOS; MARCONI, 2003).

The scientific method is based on a group of steps arranged in an orderly manner to be carried out that aim to investigate phenomena in order to obtain improvements learning (JUNG, 2009).

This exploratory research aims to refine hypotheses, authenticate instruments and enable familiarity with the field of study. It establishes the first phase of a broader study, in which it is widely used in research whose topic has been little explored, being able to be applied in initial studies to obtain an overview in relation to a certain fact (GIL, 2002).

This is basic research, *in silico in nature,* and *in vitro* with an exploratory approach, being, from the point of view of technical procedures, scientific, using as instruments for data collection, a literature review in scientific journal databases in Portuguese and English languages, developed from material already prepared, consisting of books and articles, for *in silico* analysis and discarding articles aimed at animals and *in vitro,* focusing only on adult humans. The articles researched on the topic were accessed in the Scielo databases, master's and doctoral dissertations, PubMed, in which include publications between the years 2000 to 2019.

3.1. *"DOCKING"* MOLECULAR MODELING

To carry out the tests, the digital tools PepCalc, PepDraw and Pepsite2 were used. which are found on an easily accessible and free website.

In the PepCalc tool, found on the website pepcalc.com, first type in the box entered as a sequence the code in letters, KD, then clicking on the yellow box (calculate), thus, its physical-chemical properties were obtained. For better understanding, it is possible translate the page, which originally opens in English, into Portuguese using Google's own ferment (INNOVAGEN, 2015)

In PepDraw, found at pepdraw.com, the sequence KD was initially placed on the board required, and then click on the box identified as: draw peptide. Finally, obtaining the result of its molecular shape structure and its peptide properties. Translating the page into Portuguese, for better understanding (THOMAS, 2015)

The HPEPDOCK, available at huanglab.phys.hust.edu, is initially used to perform analysis it was necessary to enter the code 1R9M, which is a Crystal structure of Dipeptidyl Peptidase IV Humana, being its FASTA Format, which is found on the RCSBPDB platform, which is a Protein Database that provides information about the 3D shapes of proteins, nucleic acids and complex assemblies. Once this is done, you must copy the code and place it in the area "Receiver input" which is located at the beginning of the HPEPDOCK tool, right after placing it if the KD sequence in the "Peptide Input" section and to finish the test click on the button "Submit" and within a few seconds the results will be displayed. Thus, with the study *in silico* the aim is to predict the binding of a given protein structure (ZHOU, 2018).

On the Pepsite2 website, initially to perform the analysis it was necessary to enter the code 1R9M after we will type the sequence KD, to obtain the interactive visualization where it showed the protein structure and a predicted peptide binding site. We then selected the PDB1R9M, to obtain the Crystal Structure of Human Dipeptidyl Peptidase IV (AERTGEERTS, *et al,* 2004)

To identify the toxicity of the KD peptide, the ferment TOXINPRED was used. available at: (https://webs.iiitd.edu.in/raghava/toxinpred/index.html).where we get the Toxicity and hydrophobicity of the KD peptide, to verify that it is not toxic to humans.

To use the PyMol software, first go to the PDB menu and select the code 1R9M of the DPP-IV enzyme, go to the FASTA option and copy the code and add it to the tool HPEPDOCK, the results will arrive in the E-mail in 24 hours, after that you must download in PDF the results, and select the 1st fitting rating - 72,967, after copying open the platform from PyMol, and select the peptide, KD, then select the enzyme 1R9M, this tool allows us to obtain the binding site as well as **the** analysis of ligand-target interactions

molecular, and the distance between the atoms, of the enzyme DPP-IV and the aspartic acid peptide and the lysine, however, cannot form hydrophobic and metallic bonds.

When the selected biological target (e.g., protein, DNA, or RNA) can whether its three-dimensional (3D) structure is known or not, a fact that determines the prioritization of planning strategies. The great advances in genomics and proteomics, combined with the evolution X-ray crystallography and nuclear magnetic resonance (NMR) techniques provide a significant increase in the number of molecular targets with 3D structures available in the Protein Data Bank (PDB). The help of methods of medicinal chemistry, it is possible to explore the immense chemical space by outlining the work of identification, selection and optimization of molecules capable of interacting with high affinity and selectivity with the selected molecular target (e.g., enzyme, receptor), which represents biological space. Various strategies can be used to investigate the chemical-biological space such as: the organization of databases, the application of filters molecular, the use of automated high-scale biological screening (HTS, from the English *high-throughput screening)* and the use of virtual screening (VS) (GUIDO; ANDRICOPULO; OLIVA, 2010).

Using binding affinity prediction method (AutoDock 4.0) to the process of position the ligand in various information in the active site of the receptor and, usually, in different conformations, in order to obtain the best interaction, identified by designation in English docking, which can be translated as “docking” or “anchoring”. This procedure allows the establishment of a classification between the compounds of greater and of lower affinity to a given receptor, having several other docking programs, among them DOCK, AutoDock, GOLD, FlexX, which perform this ordering in a automatic. The simulation **has** two procedures: the conformational search for different ligand binding modes at the receptor active site, and the evaluation of the affinity of each of these connection modes using a scoring function. In the present work, we use the PyMol program, the Pepsite2 website, HPEPDOCK, among others, which is widely used in studies of anchoring of small molecules in protein macromolecules, in addition to being free. (ALENCAR,2010).

3.2. EVALUATION OF DPP-IV ENZYME INHIBITION *in vitro*

The manufacturer of the DPP-IV kit was Enzo Life Sciences. The DPPIV Drug Discovery Kit is a complete assay system designed to screen DPPIV inhibitors, providing sufficient material to perform at least 96 assays. DPPIV (DPP4, CD26) is a member

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of the class of proteases known as prolyl peptidases, which cleave proteins after residues of proline and is believed to play roles in diabetes, cancer and autoimmune diseases, becoming a target for drug discovery. The kit contains a chromogenic substrate (H-Gly-Pro-pNA; Km = 114 µM) and a fluorogenic substrate (H-Gly-Pro-AMC; Km = 50 µM). The cleavage of p-nitroaniline (pNA) from the colorimetric substrate increases absorbance at 405 nm. The fluorimetric assay is based on the cleavage of the 7-amino-4-methylcoumarin (AMC) moiety from the C-terminus of the peptide substrate, which increases its fluorescence intensity by 460 nm. Alternative name: CD26, Dipeptidyl Peptidase IV, DPP4. Applications: detection colorimetric, fluorescent detection, HTS activity assay.

In the in vitro analysis , the amount of enzyme, substrate and peptide was determined, where the calculations were performed for the concentration of each substance. Firstly, thawed and mounted on ice; After the inhibitor was diluted 1/10, 3.5ml inhibitor /31.5ml Buffer;

Then the substrate (Gly – do/pna) was diluted – 1/50, 37ml substrate with 1813ul Buffer, with a final volume of 100ml;

After the DPP-IV enzyme was diluted in a volume (35 ml) to 0.867 ml, which was centrifuged. previously, with a stock solution of 9.97ul DPP-IV plus 490.03ul of Buffer, it was maintained in diluted or concentrated form, for several hours on ice. Because , the DPPIV enzyme is stable for at least 5 freeze/thaw cycles, to minimize the number of freeze/thaw cycles freezing/thawing, aliquot of DPPIV in tubes, was separated and store at -80 ° C. DPP-IV enzyme was not added to the blanks, only to the control/inhibitor/samples.

In this way, 0.26 ml of DPP-IV was added per well, with 10 ml of inhibitor. (diluted), and incubated for 10 minutes at room temperature;

After the start of the analysis, 50 mL of the diluted substrate (Gly – do/pna) at 37ºC was added;

Immediately after, the reading was observed every 2 minutes for a total of 10 minutes;

Then the experimental procedure was carried out, using an analysis through of the spectrophotometer technique, with this the activity of DPP-VI was determined as also the inhibition time of the KD peptide after the addition of the buffer, DPP-VI enzyme and the substrate.

At the end of the procedure, all experimental results were noted.

To determine the partial and final percentage of DPP-IV Activity, a mathematical formula, % DPP-IV Activity - In the presence of inhibitor = (Sample/ Control) x 100.

4 RESULTS AND DISCUSSION

4.1 *In silico* molecular modeling

In the studies carried out, therapeutic peptides and proteins present high specificity and efficacy at low concentrations, at this point become indispensable in the treatment of various diseases. Peptides, compared to proteins have a great advantage because they are small, they demonstrate a greater capacity to penetrate deeper into the target tissue. Peptides with therapeutic activity also present the advantage of being less immunogenic and more economical to manufacture according to the required quality parameters, related to proteins and antibodies (SOUZA, 2017).

With the discovery of bioactive molecules, medicinal chemistry also incorporates the studies of metabolism and the relationships between chemical structure and activity, making it clear that establishment of fundamental interfaces between the chemical, biological, pharmaceutical, medical, physical and computational. For studies of the evolutionary processes of molecular recognition in biological systems assumes great importance, in addition constitute the fundamental bases for understanding properties such as power, affinity and selectivity (GUIDO; ANDRICOPULO; OLIVA, 2010).

In this *in silico* study we will work with the peptide, which is made up of two residues being: Lysine (Lys-1 K) and Aspartic Acid (Asp-2 D). Presenting a pH slightly acidic. It has good solubility in water, favoring this result a good expectation of reactions, providing easy interaction with the system environment digestive.

In the Pepcalc INOVAGEN tool it was possible to have data on the physical-chemistry of the KD Peptide. Information such as molecular weight, extinction coefficient, point isoelectric, Net charge at pH 7, Estimated solubility, data shown in table 1.

Table 1: Physicochemical properties of the KD peptide

Physicochemical properties	
Number of residues:	2
Molecular weight:	261.28 g/mol
Extinction coefficient:	0 M^{-1} cm^{-1}
Isoelectric point:	pH 6.43

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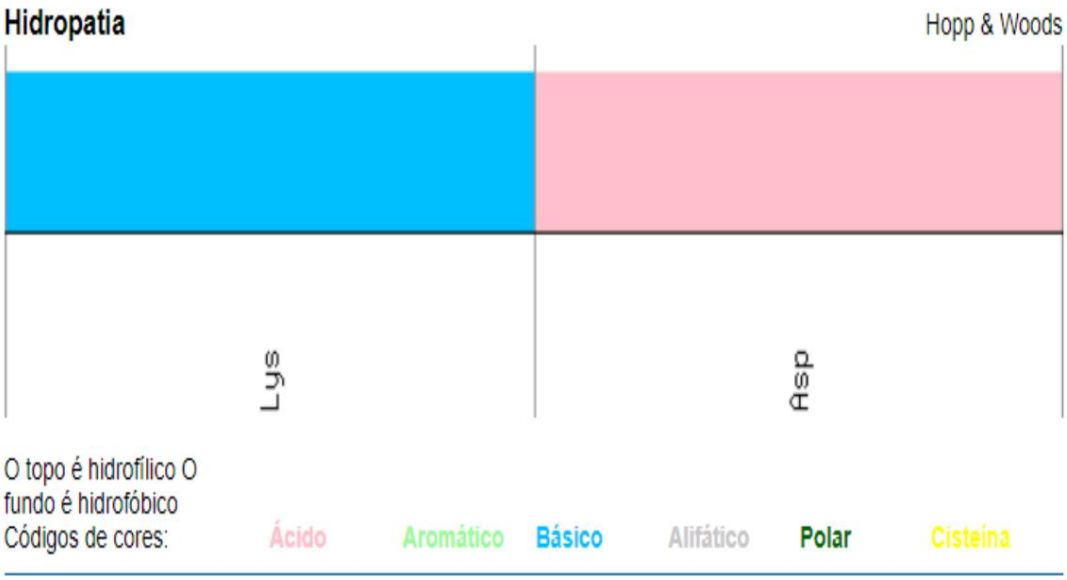
Net charge at pH 7:	0
Estimated solubility:	Good water solubility.

Source: Author's Own

Image 1: KD peptide hydropath



Source: PepCalc.com

With PepDraw it was possible to visualize the structure of the KD peptide, illustrating its peptide properties shown in Table 2.

Table 2: Peptide properties of the KD peptide

Peptide properties	
Sequence	KD
Length	2
Mass	261.1321
Isoelectric Point (pI)	6.44
Net cargo	0
Hydrophobicity	+14.34 Kcal * mol $^{-1}$
Extinction coefficient 1	0 M cm^{1} * $^{-1}$
Extinction coefficient 2	0 M cm^{1} * $^{-1}$

Source: Author himself

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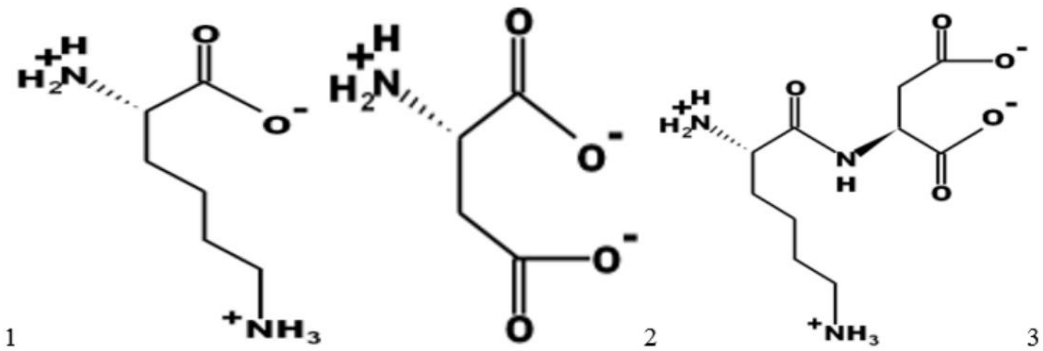
In PepDraw, you can also see the linear drawing of the peptide and reaction. condensation between the ÿ-amino group of an amino acid residue with the ÿ-carboxyl group of another amino acid residue, and with this the new peptide bond KD is formed (Image 2).

Image 2: 1. Lys-1 (K) peptide; 2: Asp-2 (D) peptide; 3. KD peptide

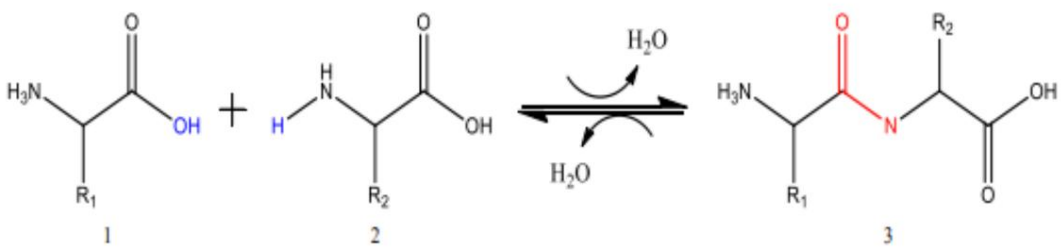


1.Molecular Formula: C6H14N2O2,2.Molecular **Formula:** C 4 H 7 NO 4,.3. **Molecular Formula:** C10H21N3O6

Source: https://www.pepdraw.com

According to the authors Nelson; Cox, 2008., peptides are biomolecules formed by union of two or more amino acids. The term “amino acid residue” or simply “residue” is used to indicate that amino acids are joined by a peptide bond. The connection peptide is formed by a condensation reaction between the ÿ-carboxyl group (1) of amino acid with the ÿ-amino group (2) of another amino acid by a covalent bond that forms a substituted amide bond Figure 2.

Figure 3: Peptide Bond



NELSON; COX, 2008

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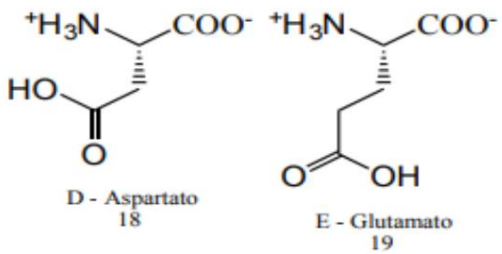
In Figure 3.4 - Formation of a peptide by the joining of two amino acid residues.
It represents the condensation reaction between the ÿ-amino group of an amino acid residue with the ÿcarboxyl group of another amino acid residue

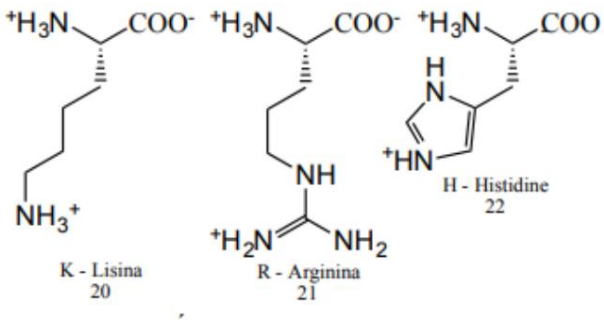
Therefore, essential amino acids are classified into 5 groups according to their radical of the side chain (R) of the ÿ-carbon, the non-polar aliphatics, the aromatics, the non-polar polar charged, positively charged and negatively charged (NELSON ; COX, 2008).

Figure 4: Negatively Charged R Group



NELSON; COX, 2008

Figure 5: Positively Charged R Group



NELSON; COX, 2008

Some peptide sequences play important roles in the systems biologicals and are commonly used as important drugs (Figure 5) (NELSON ; COX, 2008).

Table 3: Some of the main peptide sequences and their applications in biological systems

Nome	Sequência	Função
Angiotensina II	N-R-V-Y-I-H-P-F	Hormônio hipertensor.
α-Conotoxina Iml	ciclo(2-8,3-14)G-C-C-S-N-P-R-C-A-TW-R-C-NH_2	Bloqueador do receptor nicotínico de acetilcolina no sistema nervoso central.
Oxitocina	ciclo(1-6)C-Y-I-G-N-C-P-L-G	Estimula contrações uterinas e o processo de lactação.
Encefalinas	T-G-G-F-M T-G-G-G-L	Indução de analgesia em células do cérebro.
Grelina	G-S-S-F-L-S-P-E-H-Q-R-V-Q-Q-R-K-Q-S-K-K-Q-Q-P-P-A-L-K-E-P-R	Hormônio gastrointestinal estimulador da liberação do hormônio de crescimento e relacionado ao controle da ingestão de alimentos.
Vasopressina*	ciclo(1-6)C-Y-I-G-N-C-P-R-G	Ação anti-diurética.
Insulina	G-I-V-E-Q-C-C-T-S-I-C-S-L-Y-Q-L-E-N-I-C-N	Controle da glicose
Glucagon	H-S-Q-G-T-F-T-S-D-I-S-K-Y-L-D-S-R-R-A-Q-D-F-V-Q-W-L-M-N-T	

Source: NELSON; COX, 2008

There are three vasopressin analogues. Arginine vasopressin (AVP) is found in humans, lysine vasopressin (LVP) is found in hippos and phenylpressin (FVP) is found in Australian macaws and they all perform the same function.

ToxinPred presented physicochemical properties of the KD peptide, and also predicted toxicity and SVM (Support Vector Machine) score, shown in Table 3.

Table 4: Toxicity and hydrophobicity of the KD peptide

Toxicity	SVM score	Hydrophobicity	Hydropathy	Hydrophobicity
Non-toxic	- 0.80	- 0.91	-3.70	3.00

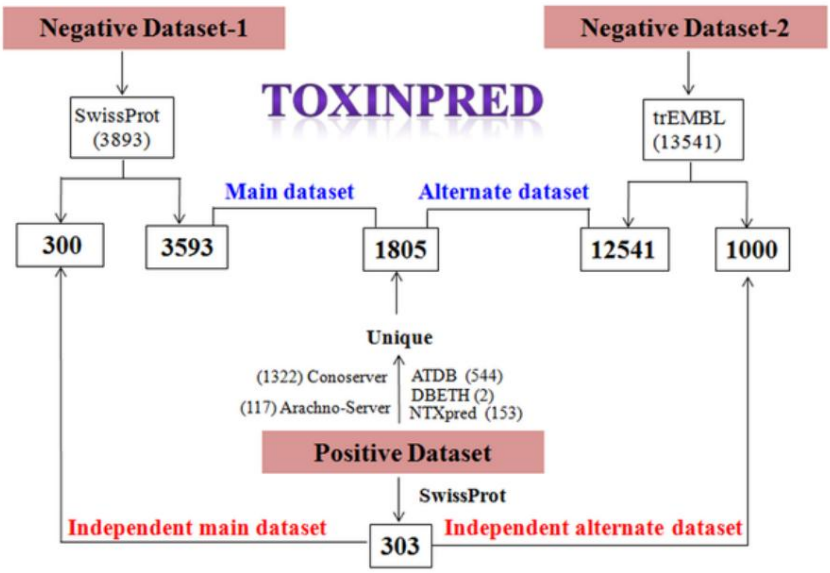
Author himself

Bringing a new drug to market can be time-consuming and costly . until its efficacy and safety are proven. **The** development of in silico approaches **facilitates** the time and expense required to bring a drug to market. Making the absorption, distribution, metabolism, excretion and toxicity (ADMET) profiles Absorption,

distribution, metabolism and excretion, predictable, obtained in a rapid process of drug discovery. All toxicological experiments aim to predict the levels of intake of substances and the possible side effects that may develop in man after its administration. For this reason, such studies are essential in investigative processes Cytotoxicity in erythrocytes is one of the experimental models of in vitro toxicity used as a screening method for substance toxicity, allowing to estimate the level of damage that can be induced *in vivo* (DIAS, 2014).

For training the main data set used and testing of SVM models was generated from experimentally validated toxic peptides (obtained from various databases) data) and well-annotated non-toxin peptides/proteins obtained from SwissProt. Includes 1805 toxic peptides as positive examples and 3593 non-toxic peptides as examples negatives. For this reason, we also generated an alternative data set, equal to the main dataset, excluding negative examples. Consists of 1805 peptides/proteins toxicants as positive examples and 12541 non-toxin peptides/proteins obtained from TrEMBL (instead of SwissProt). The above toxic and non-toxic peptides/proteins were used to produce multiple datasets for training, testing, and evaluating our models developed to predict peptide toxicity, illustrated in Figure 6. (KAPOOR *et al,* 2013).

Figure 6: to predict peptide toxicity



Source: KAPOOR *et al,* 2013

The HPEPDOCK result is presented the 10 best models in order of decreasing score, this score is shown in table 4.

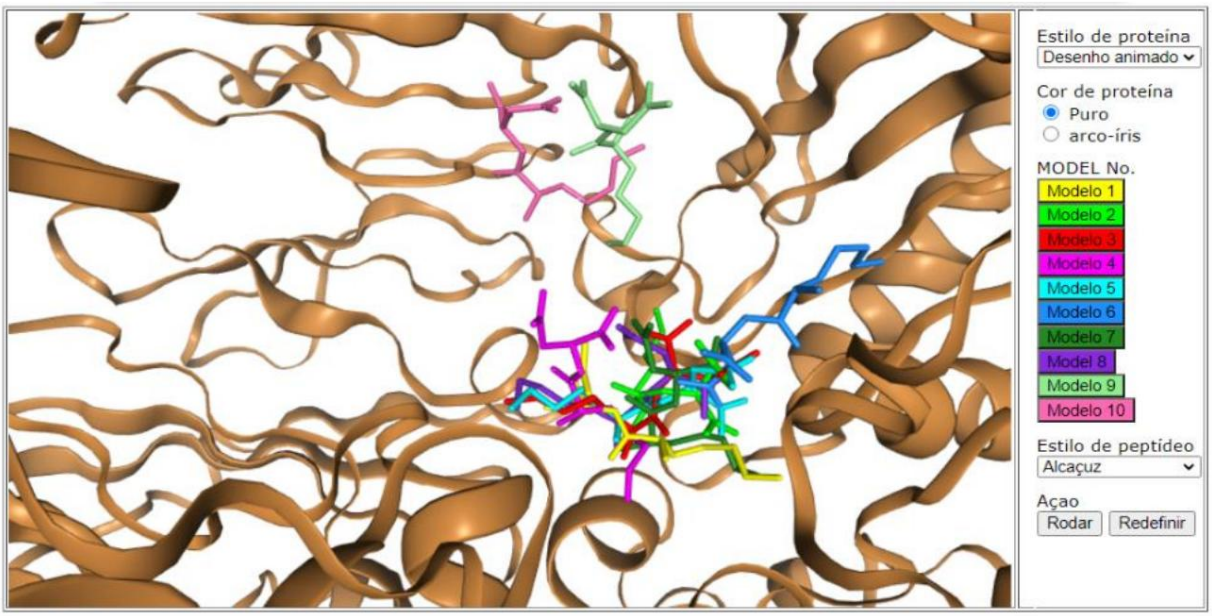
Table 5: Classification by coupling energy between KD and DPP-IV.

Classification	Fit score
1	- 72,967
2	- 65,015
3	- 63,229
4	- 63,069
5	- 62,194
6	- 61,876
7	- 61,733
8	- 61,278
9	- 60,514
10	- 59,942

Author himself

In the images it is possible to visualize the 10 positions that the KD peptide can bind to DPP-IV, with each color representing a ligament positionFigure 5.

Figure 7: 10 positions that the KD peptide



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Source: HPEPDOCK

Pepsite2 results were able to predict the binding of the KD peptide on the surface of the DPP-IV protein. The website presents the nine best positions at which the peptide can bind in the DPP-IV protein. Shown in Table 6.

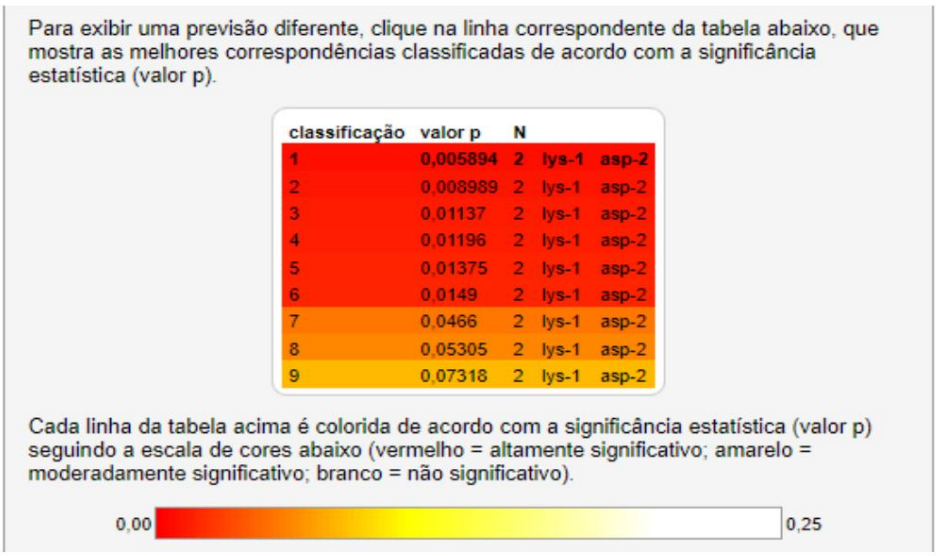
Table 6: Results of the top 6 matches ranked according to statistical significance (p-value).

Classification KD sequence	**Active amino acids P***		**Amino acids potentially bound to the enzyme DPP-4 (PDB: 1R9M)**
1st	Lys-1, Asp-2	0.005894	TRP627, TRP629, TYR752
2nd	Lys-1, Asp-2	0.008989	TRP627, TRP629, TYR752
3rd	Lys-1, Asp-2	0.01137	ARG125, GLU205, GLU206, TYR547, TYR666
4th	Lys-1, Asp-2	0.01196	TYR547, SER630, TYR631, TYR662, TYR666
5th	Lys-1, Asp-2	0.01375	TRP627, TRP629, GLY741, ALA743, TYR752
6th	Lys-1, Asp-2	0.0149	TYR48, TRP627, TRP629, HIS748, TYR752

Source: Pepsite2

In image 8, it is possible to see the results obtained in pepsite2, the classification according to with significance 1 to 9, each position is colored according to significance, the site classifies as red: Highly significant; Yellow: Moderately significant; White: Not significant significant.

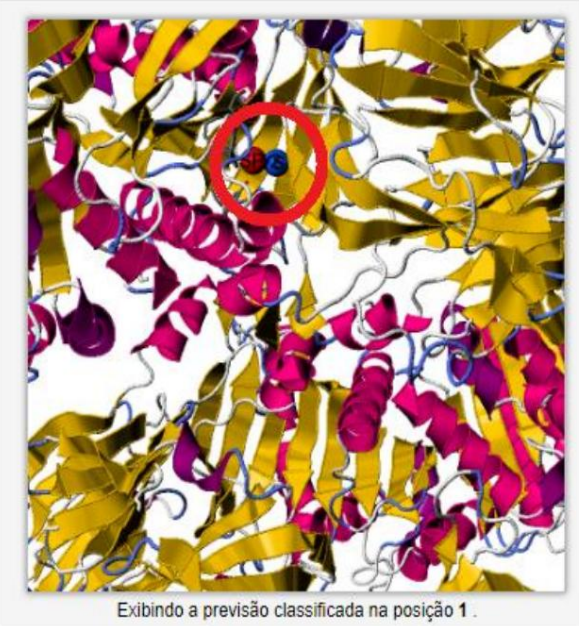
Figure 8: Classification according to significance 1 to 9



Source: Author himself

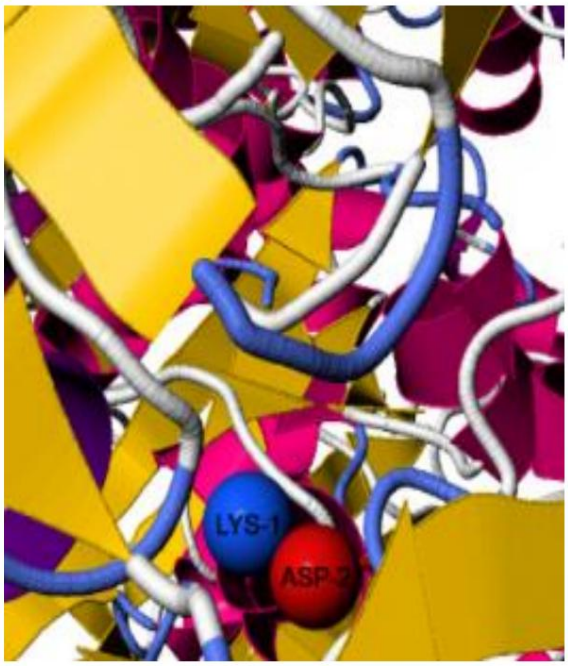
With pepste2 it is possible to visualize the Prediction of the binding of the KD peptide on the surface from PDB 1r9m protein, chain (dipeptidyl peptidase), The interactive visualization below shows the structure of the protein and a predicted peptide binding site.shows the structure in 3D of position 1 to 9 where the peptide binds to the DPP-IV protein, **(Figure 7,8).**

Figure 9: Displaying the forecast ranked at position 1



Source: Author himself

Figure 10: Displaying the 3D forecast of the team ranked in position 1

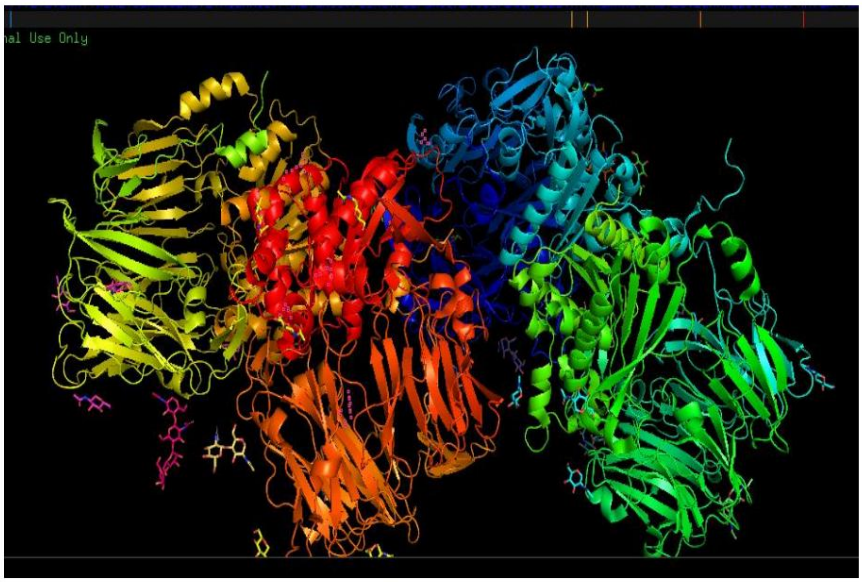


Source: Author himself

On the PyMol platform, this tool allowed us to obtain the binding site as also the analysis of ligand-molecular target interactions, and the distance between the atoms of the enzyme DPP-IV and the KD peptide (Figures 10, 11, 12). Where it is possible to observe the tertiary structure of the

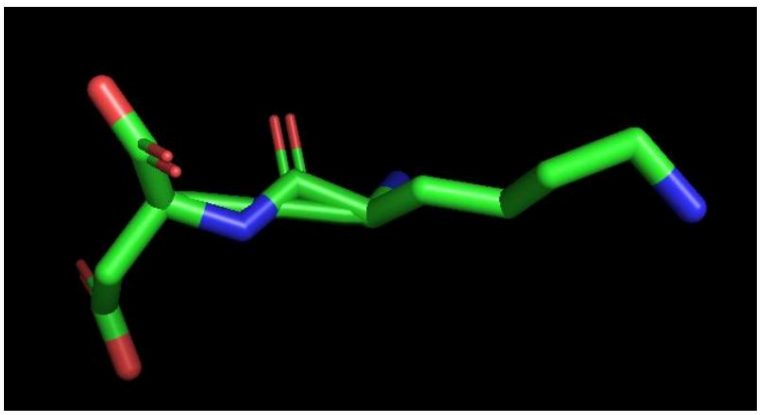
DPP-4 referring to the PDB code 1R9M, where in yellow we have the ÿ-sheets, in red the ÿ-helices and in green the loop region, the hydrophobic potential surface can be seen

Figure 11: Represents the surface of the 1R9M enzyme



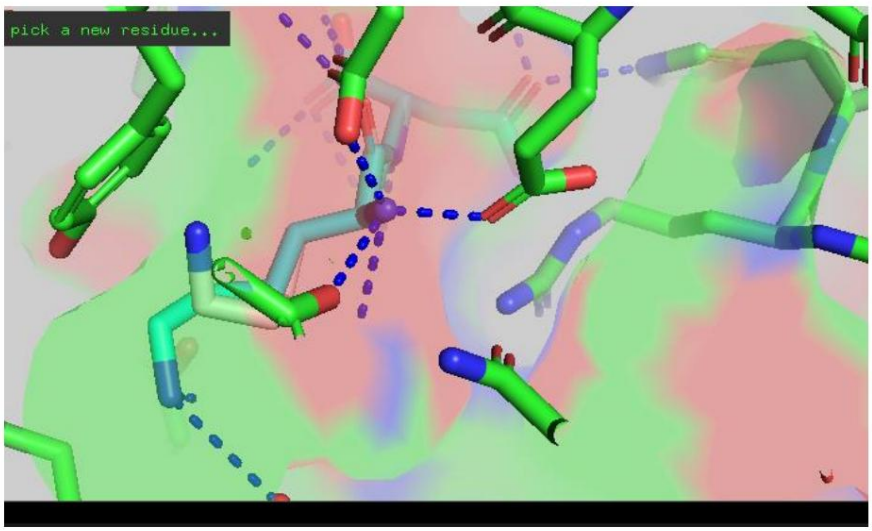
Source: Author himself

Figure 12: Represent the surface of the KD peptide



Source: Author himself

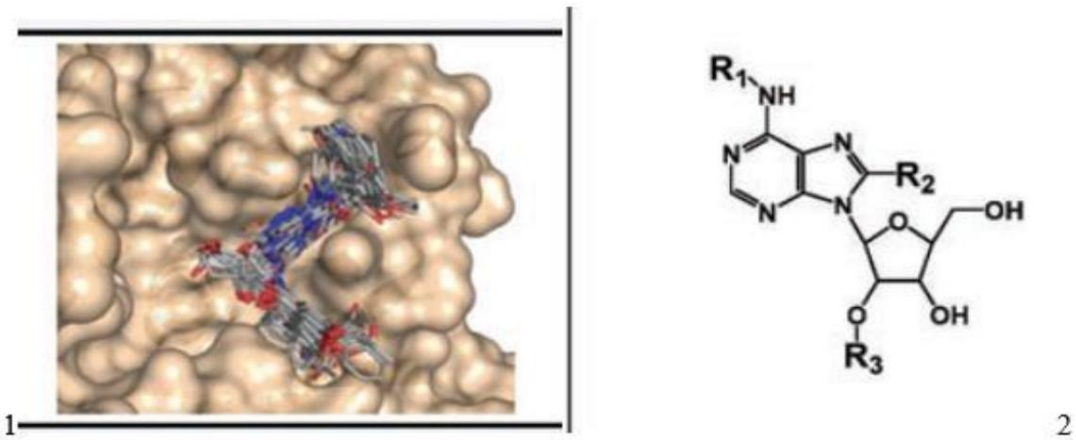
Figure 13: Ligand-molecular target interactions



Source: Author himself

Using the dataset in the generation of 3D QSAR models, (Quantitative Structure-ActivityRelationship) applying mathematical and computational models to chemical structure of substances to predict, qualitatively or quantitatively, physicochemical properties, toxicological and ecotoxicological, which serve to evaluate possible adverse effects (TAVARES, 2004), (Figure 13) informing the predictives was made up of 70 derivatives of adenosine and the General structure of the series of LmGAPDH inhibitors, adenosine derivatives, which were designed based on the significant structural differences between the active sites of enzymes of the parasite *Leishmania mexicana* and its human counterpart. Thus, the properties molecular were quantitatively related to inhibitory potency, using the methods of comparative analysis of molecular fields(GUIDO; ANDRICOPULO; OLIVA, 2010).

Figure 14:1, Structural alignment of the 70 inhibitors; 2, General structure of the series of LmGAPDH inhibitors, adenosine derivatives.



Source: GUIDO; ANDRICOPULO; OLIVA, 2010

Holograms can be graphically converted into maps using a color scale specific, with the green and yellow colored regions indicating fragments that contribute positively to biological activity; areas in red and orange contribute negatively for this parameter. Thus, the HQSAR method allows a visual inspection of the regions of the molecule that can/should be preserved. Molecular anchoring is a in silico method that predicts the orientation of a compound (ligand) in the possible active site of the target biological, forming a stable complex between ligand/receptor. This technique evaluates this forms the fit of the molecule considering the interaction energy and its geometry, which can be considered an important tool in Medicinal Chemistry, especially in the projection rationale for new drug candidates. Several studies were found in the literature and mentioned the use of the molecular anchoring technique considering only the structure of the catalytic triad as flexible and hydrogen bonds and hydrophobic interactions of the triad catalytic, in the case of proteins such as DPP-4, which would contribute exclusively to the binding of the substrate to the enzyme binding site, (Figure 14) Demonstrates the schematic representation of how the HQSAR technique works, (PANTALEÃO, 2014).

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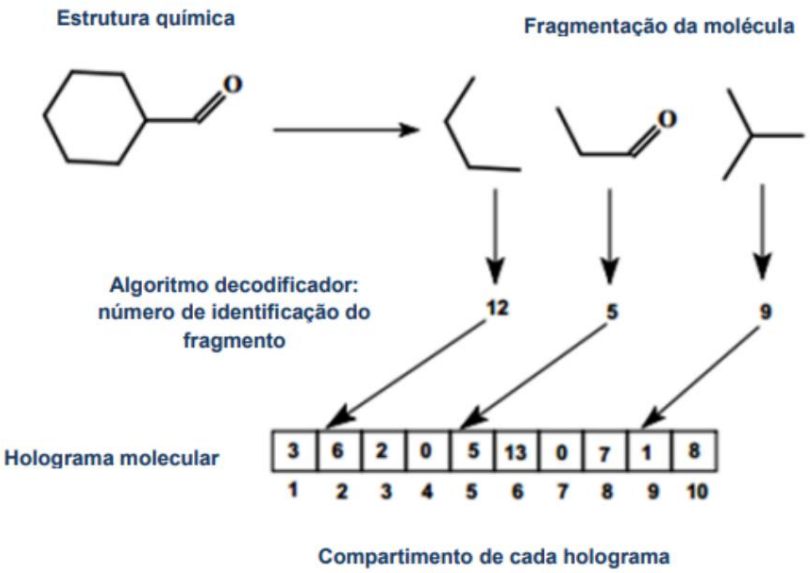
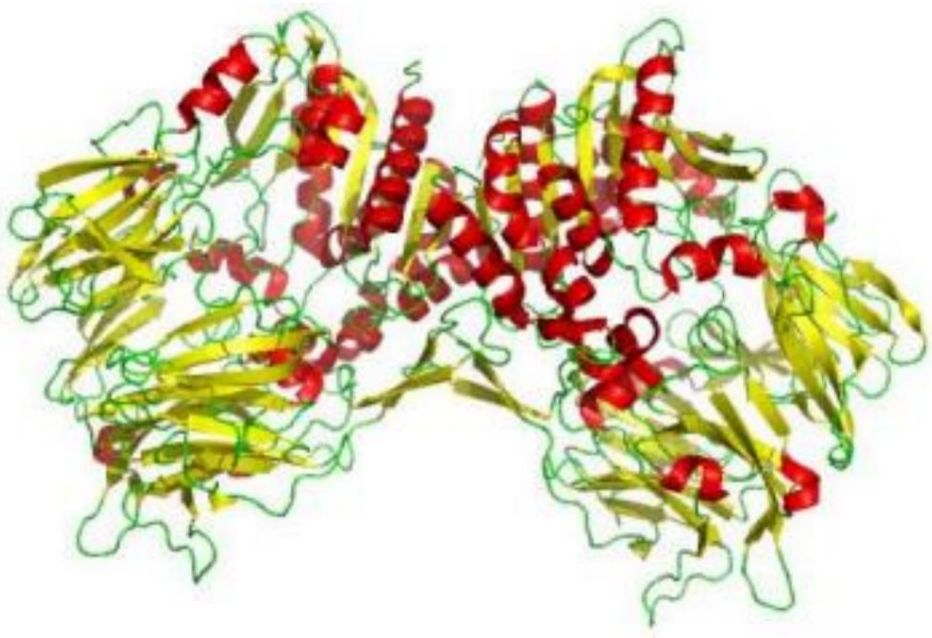
Figure 15: Schematic representation of how the HQSAR technique works



Source: (PANTALEON, 2014).

From Figure 14, it is possible to observe the tertiary structure of DPP-4 referring to PDB code 4A5S, where the ÿ-sheets are in yellow, the ÿ-helices are in red and the loop region is in green, the hydrophobic potential surface can be seen. (PANTALEON, 2014).

Figure 16: Structure of DPP-4 (PDB 4A5S) 2



Source: (PANTALEON, 2014).

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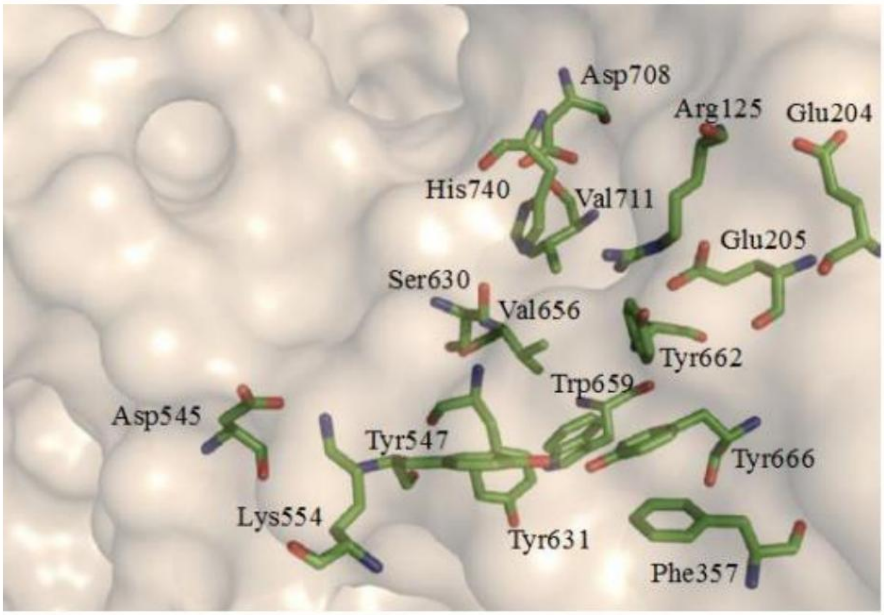
Next, in (Figure 16), you can see the arrangement of the amino acids that make up the active site of DPP-4 enzyme

Figure 17: Representation of the active site of the enzyme DPP-4 (PDB 4A5S 25,26), indicating the main amino acids that make up the catalytic region.



Source: (PANTALEON, 2014).

The results obtained in HPEPDOCK, classification 1 shown in table 3, indicates that the lower the fit score value, the better the energy score will be. coupling. Thus, the score of (-72.967) has a better result. Meaning that in this position the KD peptide was shown to be capable of binding to the active site of the DPP-IV receptor. Thus, like HPEPDOCK, Pepsite2 and PyMol also indicated that binding is possible between the KD peptide and the DPP-IV enzyme, the tool shows the potentially amino acids linked in the DPP-4 enzyme as in the case of position 1 with the P* value of (0.005894) in this position the KD peptide binds to three amino acids of the D chain, namely TRP627, TRP629 and TYR752. This position is also shown in the table, with the color red, which indicates that the interaction of the KD peptide with DPP-IV is highly significant.

4.2 *IN VITRO* ANALYSIS

In *in vitro* studies , therapeutic peptides also demonstrate several advantages, compared to the small organic molecules that make up traditional drugs, a since they are often made up of the smallest functional part of a protein, distributing

greater efficacy, specificity and selectivity. In the studies of the results peptides present fewer drug interactions, because the peptide degradation products are made up of amino acids. Thus, given that they have a short half-life, few peptides accumulate in the tissues, reducing the risk of complications caused by its metabolites (SOUZA, 2017).

With ongoing studies a large production of countless peptides biologically active substances have been synthesized in the last fifty years. In the vast majority of productive parts refers to synthetics obtained by chemical methods (in solution or in phase solid), enzymatic or combination of both (semi-synthesis) in carrying out scientific research in different areas, for therapeutic and food use, for vaccine production or even as blocks constructive of other molecules with biological action. For this reason, studies and production in pharmaceuticals, synthetic peptides began to serve as unequivocal proof of identities chemical and biological roles of natural peptides. (MACHADO *et al,* 2004)

During the experiment, all the basic requirements related to to the performance of the method and equipment involving: accuracy, recovery, robustness, accuracy, reaction blank measurement, sensitivity, detection limit, interference and value of reference.

Firstly, to determine the concentration of each substance, a calculation was made mathematical, (C . V2= C . V), to obtain sample concentration, Buffer, DPP-IV and substrate, and with the tip of a pipette all the substances were added to a slide to analysis through the spectrophotometer technique, as the analysis progressed it was necessary to do some adjustments, in which the inhibitor in the first replicate was added 5u/l and at the end was 35 u/l for a 0.7 u/l cuvette and in the buffer it was zero, in the second replicate the inhibitor was 35 u/l to 17.5 u/l as well as the Buffer, and the cuvette of 3.35 u/l.

That way , In this *in vitro* study, the enzyme activity was accurately analyzed in Presence of Peptide KD, after analysis of the control, without the inhibitor reaction, and with the reaction of inhibitor, then the mean and standard deviation of each of them were calculated using a mathematical calculation.

To identify the percentage of DPP-VI activity as well as the inhibition time of the KD peptide after the addition of the buffer, DPP-VI enzyme and the substrate, a analyze using the spectrophotometer technique, where the transmittance values are determined (transmitted light) and absorbance (absorbed light) of a solution at one or more wavelengths wavelength. It measures the amount of photons (the intensity of light) absorbed after passing through sample.

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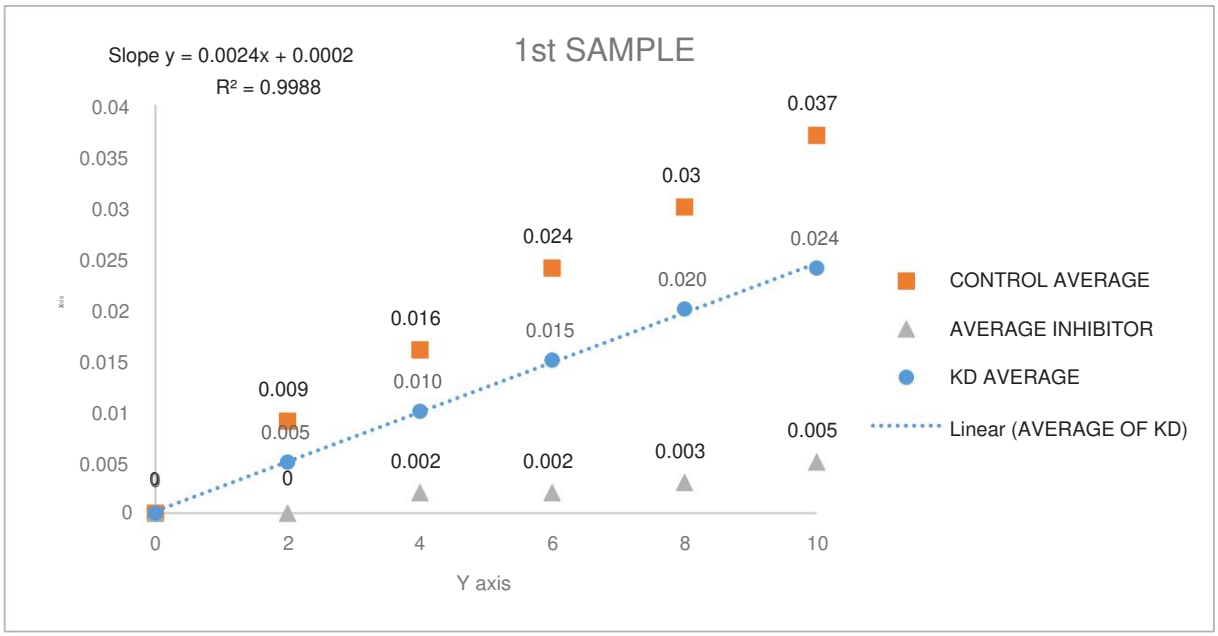
The control was of great importance, as it allowed the quantification by comparison of the results obtained from DPP-VI activity. The modifications were confirmed in analyses of spectrophotometer.

In enzymatic or biocatalyzed synthesis, the formation of the peptide bond does not occur by a chemical reagent, but rather by an enzyme in its free or immobilized form. In the same way As occurs in chemical synthesis, enzymatic synthesis may require several steps during the process of total synthesis (MACHADO et al., 2004).

Therefore, it was a great satisfaction to replicate the selected samples, as determined the final positive result, in (Graph 2), illustrates the slope of the reaction mean of the inhibitor of the activity of the KD peptide on the DPP-IV enzyme in a given time, compared with the control average, so we can see that the slope point of the peptide is closer to the average of the control.

Quality control is defined as a concept, such as techniques and activities operational methods used to monitor the implementation of the specified quality requirements. The external quality control, also includes the evaluation of the performance of systems analytical through proficiency testing, analysis of certified standards and comparisons interlaboratory. Internal quality control are also procedures conducted in set of samples in order to verify that the analytical system is operating within the limits pre-defined tolerance levels (SANTOS APS; ZANUSSO JUNIOR, 2015).

Graph 1: Slope of control, inhibitor, and KD.



Source: Author himself

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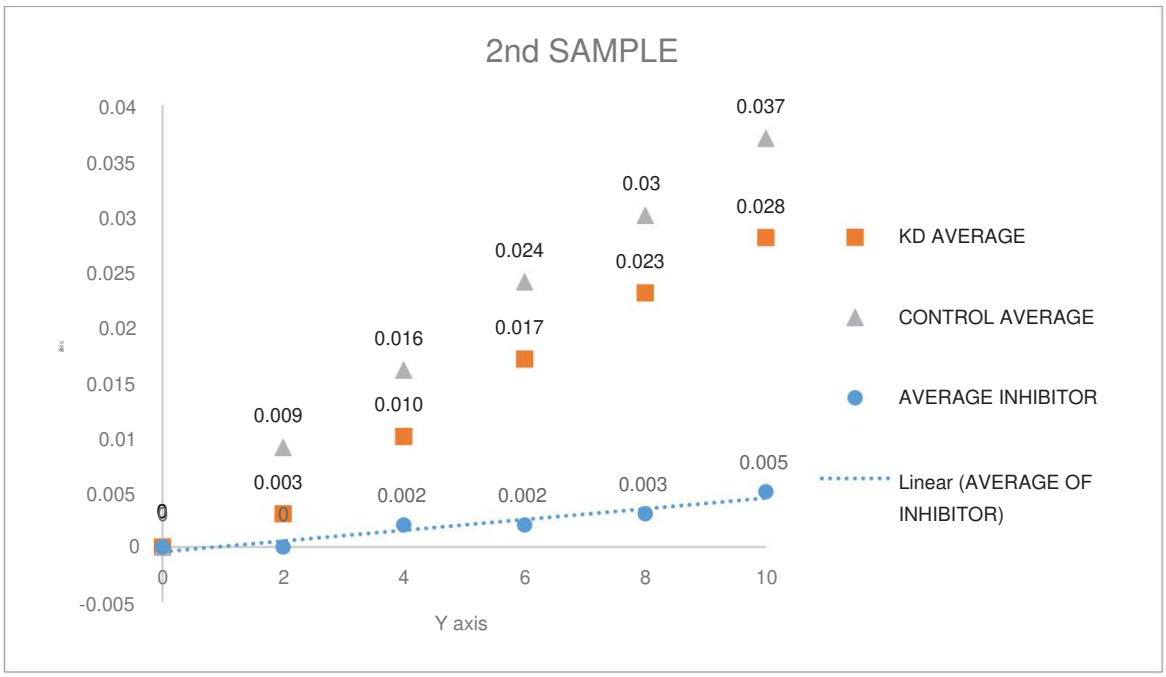
In this second sample, the graph also keeps the enzyme activity apart from the time after 6 minutes, however the inhibition was lower compared to the first sample, it should may have been due to the reduced quantity of samples. Because in the second replicate the inhibitor was 35 u/l to 17.5 u/l as well as the Buffer, and the cuvette of 3.35 u/l, (Graph 3).

It was identified that a "optimized sampling" is not obtained based on only the value judgment and practical experience of the collector, but rather the greater the precision required, the higher the cost involved. Some sampling and analysis errors exist in some moment, and must be balanced against each other in relation to the intrinsic value of the material, mainly in relation to the cost arising from the consequences of errors. Changing a sample or characteristic to be analyzed, for example, when the parameter of interest is the humidity, and the collector leaves the sample exposed to a source of heat or humidity, can influence the final result (GÓES; LUZ; POSSA,2004).

Graph 2. Slope of control, inhibitor, and KD



Source: Author himself

In this first sample, the graph shows the enzymatic activity apart from time. after 6 minutes, when it reached 10 minutes there was a considerable inhibition of the DPP-VI activity, as it obtained a partial result of 64.8% and a final result with a inhibition of 35%. However, the results of the second sample were not accurate compared to the first

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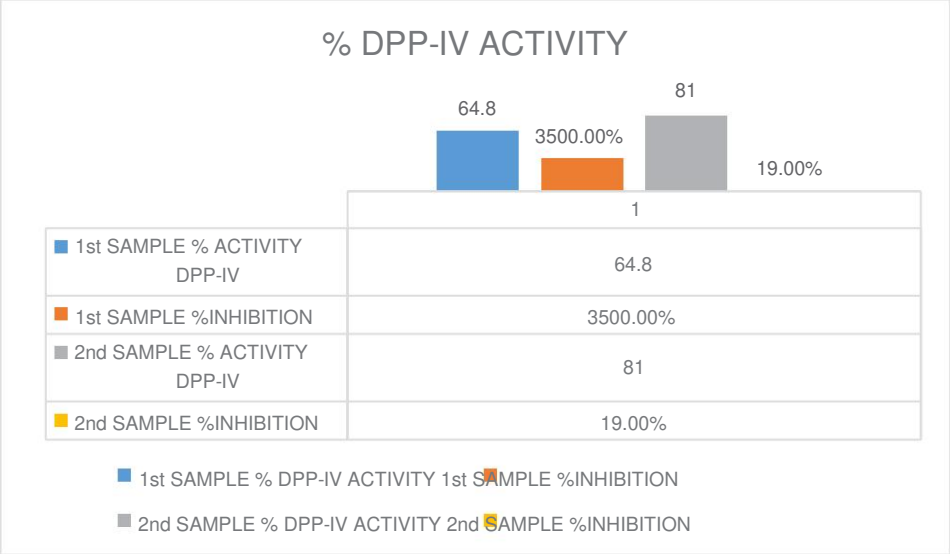
sample, as the partial results were 81% and the final result with an inhibition of 19% inhibition of DPP-VI activity (Graph 4).

Graph 3. % of DPP-IV Activity.



Source: Author himself

This methodology offers advantages over chemical synthesis, as well as high stereo; absence of racemization and other side reactions typical of synthesis chemistry; partial protection of substrates; tolerance to the presence of water in reaction media; possibility of using reactors for large-scale production at lower costs. On the other hand, hand, the fact that there is no universal enzyme means that the methodology is not general or applicable to any peptide sequence (MACHADO *et al.,* 2004).

Because the formation of a peptide bond catalyzed by an enzyme is based on simple inversion of the hydrolysis reaction, aminolysis of amides (also called transpeptidation) and aminolysis of esters. The reversal of hydrolysis and aminolysis of amides are reversible and therefore thermodynamically controlled. Aminolysis of esters is irreversible and can therefore be considered kinetically controlled (SOUZA, 2017).

In the *in vitro* study, the graphs demonstrate the enzymatic activity apart from the time of after 6 minutes, when it reached 10 minutes there was a considerable inhibition of DPP-VI activity. The results of the first sample were accurate, as they were well close to the minimum control value.

Thus, with *in silico* studies , there was binding of the KD peptide with the DPP-IV enzyme, therefore, in vitro analyses obtained a significant inhibition of DPP-IV with the addition of the peptide.

Based on these positive results, there may be further studies to prove 100% of the inhibition of DPP-IV activity.

Having become the study of bioinformatics and chemoinformatics increasingly necessary for exploring the pharmacological potential of substances, through selection of anchoring targets, as an example, to identify relationships between the actions revealed in the targets and therapeutic effects. *In silico* models are used as a procedure for screening, identifying the effect of a drug stimulus on cells, tissues, guiding experimental research and clinical studies, however they are theoretical data until to be validated. *In silico* models are useful, but they lack the biophysical characteristics humanities (DIAS, 2014).

5 CONCLUSION

It is concluded that in *silico analysis,* with the use of all these tools, it was possible highlight the very relevant results. With all these results of the peptide interaction KD extracted from cowpea, with the active site of DPP-IV, shows that there is a great possibility inhibition of the DPP-IV protein through the KD peptide. Enabling GLP-1 to increase the insulin secretion, improving glycemic control in patients with type 2 diabetes *mellitus* 2. And through in *vitro analysis,* it was possible to observe the inhibition of the activity of the KD peptide after a certain time, in view of these positive results, more should be done *in vitro* tests so that synthetic peptides can be developed in the correct sequences. This essential factor for obtaining reliable results in the evaluation of tests research. Furthermore, in the clinical setting, the purity of the drug or supplement is a critical issue. mandatory, required by competent regulatory units.

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