In silico pharmacokinetic and toxicological study of Cinnamic Acid analogues

Estudo farmacocinético e toxicológico *in silico* de análogos do Ácido Cinâmico

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ABSTRACT

Cinnamic acid analogs are natural phenolic compounds that have a wide range of biological and therapeutic activities. The present work aimed to predict, through in silico methodologies, the oral bioavailability and pharmacokinetic and toxicological analyzes for four cinnamic acid analogues (caffeic acid, ferulic acid, p-coumaric acid and synaptic acid). The study revealed that the analogues have good oral bioavailability, favorable pharmacokinetic and toxicological parameters. The *Virtual Screening* performed to predict oral bioavailability indicated that all analogues do not violate Lipinski's Rule. The *in silico* ADME study of pharmacokinetic parameters showed that all derivatives have high intestinal absorption, are permeable by Caco-2 cells, do not cross the blood-brain barrier, do not inhibit P-glycoprotein. There will be no inhibition of the cytochrome P450 complex isoenzymes (CYP450). The *in silico* Toxicological study revealed that the analogues do not have toxicity by the AMES Test, are not carcinogenic and do not present acute oral toxicity.

Keywords: natural products, Phenylpropanoids, Cinnamic Acid, chemoinformatics, *in silico* ADME, *in silico* toxicology.

RESUMO

Os análogos do ácido cinâmico são compostos fenólicos naturais que possuem ampla gama de atividades biológicas e terapêuticas. O presente trabalho objetivou predizer, por intermédio de metodologias *in sílico*, a biodisponibilidade oral e análises farmacocinéticas e toxicológicas para quatro análogos do ácido cinâmico (ácido caféico, ácido ferúlico, ácido p-cumárico e ácido sináptico). O estudo revelou que os análogos apresentam boa biodisponibilidade oral, parâmetros farmacocinéticos e toxicológicos favoráveis. O *Screening Virtual* realizado para a predição da biodisponibilidade oral indicou que todos os análogos não violam a Regra de Lipinski. O estudo ADME *in silico* de parâmetros farmacocinéticos, indicou que todos os derivados apresentam elevada absorção intestinal, são permeáveis pelas células Caco-2, não atravessam a barreira hematoencefálica, não inibem a glicoproteína P. Não haverá inibição das isoenzimas do complexo citocromo P450 (CYP450). O estudo Toxicológico *in silico* revelou que os análogos não possuem toxicidade pelo Teste de AMES, não são carcinogênicos e não apresentam toxicidade aguda oral.

Palavras-chave: produtos naturais, Fenilpropanóides, Ácido Cinâmico, quimioinformática, ADME *in silico*, toxicologia *in silico*.

1 INTRODUCTION

Natural products are chemical species produced by microorganisms and plants with various functionalities, among which one can mention the formation of protective barriers in the human organism and the defense against microbial infections. Natural products have relevance in the therapy of several pathologies that afflict the human being, therefore, are considered the object of research in the discovery, development and optimization of promising drugs (JOHN, 2010). Between 1981 and 2002, several drugs derived from natural products were developed against different categories of cancer (RODRIGUES, 2015). There are also reports in the literature of drugs developed in the treatment of diseases such as Alzheimer's disease and diabetes Mellitus (LAM, 2000).

The importance of natural products is evidenced in several research studies for the development of new drugs. In this sense, one of the prominent categories refers to phenolic compounds of the phenylpropanoid class, commonly classified in pharmacognostic terms as Cinamic Acid (CA) analogs: caffeic acid, ferulic acid, p-cumaric acid, and synapic acid (SIMÕES, *et al*., 2007). The derivatives of cinnamic acid present in the chemical structure the basic skeleton (C6-C3) with at least one aromatic ring and one hydroxyl group (MICHALAK, 2006).

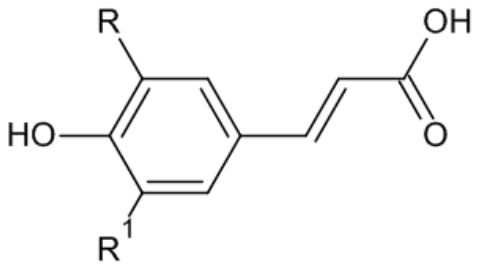
The analogs of AC, are classified as compounds natural products widely distributed in the plant kingdom, i.e. practically all plant tissues possess at least one of the mentioned derivatives. They are found in foods of the human diet and responsible for taste, odor, pigmentation, among which stand out: coffee, blueberries, cherries, plums, apples, kiwis, grapes, green tea, spinach and cinnamon (SHAHIDI *et al.*, 2004). Cinnamic acid is also found in red wine and citrus fruits, while ferulic acid in the external portions of cereal grains, such as corn flour, whole wheat, oats, and rice (PRASAD *et al.*, 2010).

Research has revealed that cinnamic acid acts by inhibiting the DNA synthesis of growing cells (DE *et al.*, 2011). Most AC analogs are able to bind to the lignin-polymer complex, to the hemicellulose of the plant cell wall, conferring mechanical resistance, chemical and biological resistance, against bacteria and phytopathogens. They are also able to modulate enzymatic activity, resulting in numerous benefits to the human organism (GALLÃO, 2012).

Caffeic acid is widely found in various foods to inhibit the growth of the bacterium *Clostridium botulinum* (FU *et al*, 2010). It is noted in the literature that caffeic acid has antioxidant biological activity (NIJVELDT, *et al.*, 2001), anti-hypertensive, anti-fibrotic, anti-tumorigenic (PRASAD,et al.2010) and antiviral (ZHANG, et al., 2007). Ferulic acid has important antioxidant activity, being used as an anti-inflammatory and acts in the fight against free radicals, through the production of cytoprotective enzymes and through the inhibition of the nitric oxide synthase enzyme (BATISTUZZO *et al.*, 2015). P-cumaric acid is commonly found in spinach, beet, cumin seeds, and cereals. It has considerable biological activity as antioxidant, antitumor, anti-inflammatory and antimicrobial (CLIFFORD, 2000). The bactericidal activity of p-cumaric acid is justified by two mechanisms: 1) Increased permeability of the bacterial cell wall and by loss of barrier function; 2) Occurrence of chemical binding of acid to the phosphate radical in the double helix of deoxyribonucleic acid (DNA), inhibiting the cell multiplication of microorganisms (LOU, 2012). Synapic acid, on the other hand, has two closely related methoxy groups, allowing the derivative to present antioxidant activity much higher than that of ferulic acid and lower than that of caffeic acid (NETO, 2021).

The two-dimensional (2D) chemical structure of the phenylpropanoid prototype and its analogs are shown in Figure 1.

Figure 1: Two-dimensional (2D) chemical structure of the phenylpropanoid prototype and its analogs.



R	**=**	**R**	**=**	**OCH_3**	**R**	**=**	**R^1**	**=H**	**R**	**=**	**R^1**	**=**	**OCH_3**
OHR1	**=**	**R^1**	**=**	**Ferulic**									
Caffeic acid		acid			**p-cumaric acid**				**synapic acid**				

Source: ChemSketch® Freeware version 2021.

Although a variety of *high* yield in *vitro* screening methods are widely used, it is still considered to be difficult to obtain AMDETox data from compounds (HOU and WANG, 2008). Studies using *in silico* methods show that prediction of chemical absorption, distribution, metabolism, excretion and toxicity (ADMETox) profiles play a significant role in the discovery of new drugs (YANG *et al*., 2018).

Chemoinformatics is a multidisciplinary area capable of providing relevant advances by means of the use of computer science resources for forecasting various molecular descriptors. This work used the databases of the following platforms: Molinspiration Cheminformatics® (https://www.molinspiration.com) (GROB,1986) and admetSAR® (http://lmmd.ecust.edu.cn/admetsar2/). The objective of the present study was to perform the analysis of the oral bioavailability profile and the pharmacokinetic and toxicological study *in silico* for the four cinnamic acid analogs: caffeic acid, ferulic acid, p-cumaric acid and synaptic acid.

2 MATERIAL AND METHODS

In this research, computational programs and online databases of Chemoinformatics were used to determine the molecular descriptive properties of the chemical structures of the four (04) analogs derived from cinnamic acid: caffeic acid, ferulic acid, p-cumaric acid and synaptic acid.

2.1 MOLECULAR MODELING

Initially, the chemical structures of the cinnamic acid analog molecules were designed two-dimensional (2D), visualized three-dimensional (3D) using the computer

program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the energies (E_1) of the three-dimensional chemical structure of all analogs were tabulated. Subsequently, Energy Minimization for all derivatives was performed using the semi-empirical Quantum Method PM3 (Parametric Method 3) with the aid of the Arguslab® Freeware program version 4.0 (Thompson and Planaria software LLC, Inc., 1997) and the steric energies (E_2) of the chemical structures of the analogs were tabulated. In order to obtain the local minima of the chemical structures of the cinnamic acid derivatives, geometric optimization was performed using Density Functional Theory (DFT), via BLYP hybrid method (Becke, Lee, Yang and Parr) using the base function 6-31G (d,p) simultaneously with the Simplex Method to simulate the analogs in aqueous environment using dielectric constant (ε = 78,4) with the aid of Molecular Modeling Pro Plus® (MMP Plus®) program version 8.0 (ChemSW, Inc,. 2017). Finally, all optimized chemical structures were saved in MDL-type molfiles (.mol) and the steric energies (E_3) of all optimized chemical structures were tabulated to be used in later studies.

2.2 PHARMACOKINETIC STUDY *IN SILICO* HUMAN FOR ORAL BIOAVAILABILITY

With the help of computer programs and the source code of the file (.mol), the code SMILES (*Simplified Molecular Input Line Entry Specification) was obtained* and exported to the online database platforms. Through Chemoinformatics, the study was carried out *in* human *silico pharmacokinetics* to predict descriptive molecular properties for all analogs with the help of the Molinspiration Cheminformatics® database (https://www.molinspiration.com) (GROB, 1986). This database predicts molecular descriptors to evaluate the oral bioavailability of molecules taking into account Lipinski's Rule, also known as the *Rule of Five*.

2.3 PHARMACOKINETIC STUDY *IN* HUMAN *SILICO* (ADME *IN SILICO*)

Initially, the ADME *in silico* study (ADME) was *carried out* for the *absorption, distribution, metabolism and excretion* analogs of cinnamic acid, with the aim of predicting the following molecular parameters: Human Intestinal Absorption (HIA), Permeability through the Haematoencephalic Barrier (BBB), Inhibition of P-glycoprotein and Cell Distribution of derivatives in the human organism. Subsequently, the ADME *in silico* study was performed to predict inhibition and interaction with hepatic cytochrome

P450 complex enzymes (CYP450) in the process of Hepatic Metabolization (Hepatic Biotransformation) of cinnamic acid analogs. The ADME *in silico* study was conducted with the assistance of the Chinese admetSAR® online platform (http://lmmd.ecust.edu.cn/admetsar2/), coordinated by Professor Dr. Yun Tang, Leader of the Molecular Modeling and Design Laboratory (LMMD), of the School of Pharmacy of the University of Science and Technology of Eastern China (YANG, et al., 2018).

2.4 TOXICOLOGICAL STUDY *IN* HUMAN *SILICO*

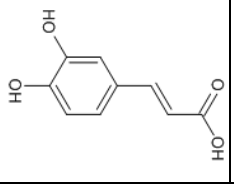
The human *in silico* toxicology study for cinnamic acid analogs was conducted to predict AMES toxicity (T: toxic; NT: non-toxic), carcinogenicity (C: carcinogenic; NC: non-carcinogenic), and Acute Oral Toxicity in Categories. The present study was also carried out with the help of the Chinese platform admetSAR® (http://lmmd.ecust.edu.cn/admetsar2/) (YANG, *et al¹., 2018).*

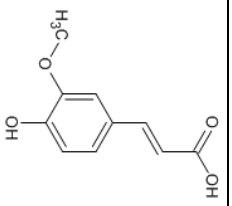
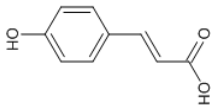
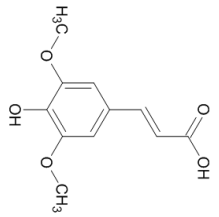
3 RESULTS AND DISCUSSION

3.1 MOLECULAR MODELING

Energy minimization and optimization of molecular geometry are the processes in which the atomic coordinates of the molecule are altered in order to reduce its steric energy corresponding to its local minimum (SANT'ANNA, 2009). After carrying out the Molecular Modeling stage (2D Design, 3D Design, Energy Minimization and Geometric Optimization) for the chemical structure of the derivatives of cinnamic acid, all the optimized chemical structures were saved in files of the type MDL molfiles (.mol) and the steric energies (E_3) were tabulated. Table 01 represents the two-dimensional chemical structure (2D) of each cinnamic acid analog and their respective energies E_1, E_2 and E3 in Kcal/mol of the local minima of each chemical structure after the above steps have been performed.

Table 1: 2D Chemical Structure of the analogs and values of the steric energies E_1, $E_{2\text{ and }E34}$.

Analogs	**Two-Dimensional Drawing (2D)**	**E_1 (Kcal/mol)**	**E_2 (Kcal/mol)**	**E_3 (Kcal/mol)**
Caffeic Acid		55.5666	21.7682	18.6248

Ferulic Acid		63.3853	21.9511	19.8568
p-coumaric acid		54.9607	19.7477	12.9934
Synapic Acid		71.8180	24.3617	20.9915

Source: ChemSketch® Freeware version 2021, Arguslab® Freeware version 4.0 and MMPplus® version 8.0.
E1: Steeric Energy (3D Drawing); E2:Energy after Energy Minimization and energy minimization after Geometric Optimization.

Analysis of table 1 shows that the hybrid method used (Energy Minimization: PM3 + Geometric Optimization: DFT) has considerably reduced the steric energy of the chemical structure of all cinnamic acid analogs.

3.2 PHARMACOKINETIC STUDY *IN SILICO* HUMAN FOR ORAL BIOAVAILABILITY

The assessment of the oral bioavailability profile of the compounds was performed using the Chemoinformatics Molinspiration Cheminformatics® platform (https://www.molinspiration.com). The oral bioavailability profile takes into account the Lipinski Rule, also known as the *Rule of Five*. Researcher Christopher Lipinski developed the Rule of Five based on the hypothesis that several molecular properties (molecular descriptors) obtained for the vast majority of compounds that are in the preclinical stages and in the safety evaluation of phase I were discarded in the drug development process (LIPINSKI *et al.*, 2004).

The rule predicts whether or not a compound has promising properties to be a drug. In order for a drug to be developed, it must have favorable physicochemical properties, that is, it will be based on the *United States Adapted Name* (USAN), the *International Non-proprietary Name* (INN) and the immense databaseWord Drug Index

(WDI) *to be evaluated later in the safety phase II (PANDIT, 2008).* The study will indicate whether the analog will obey Lipinski's Rule and estimate whether the molecules of the compound present a high rate of human intestinal absorption, plasma solubility, in tissue liquids and, permeability by biological membranes when administered orally.

Lipinski's rule takes into account the following criteria: Molecular Weight (PM), which should not exceed 500 Da (Dalton); Log P = miLogP (logarithm of the partition coefficient), whose limit value is 5; Hydrogen Link Donor Sites = SDLH and Hydrogen Link Acceptor Sites = SALH, which should not exceed respectively the values 5 and 10 and the Polar Surface Area (TPSA = *Polar Surface Topological Area*) which should be less than 140 ($Å^2$) These parameters account for 90% of the drugs administered orally that are at the stage of phase II clinical development (LIPINSKI *et al*., 2004). Other descriptors such as the Number of Rotatable Links (NLR) and Molecular Volume (VM) were determined as an extension of Lipinski's rule. The extension of Lipinski's rule, known as Veber's rule, indicates that molecular flexibility, as a measure of rotatable bonds, can have a considerable influence on oral bioavailability, i.e., the greater the molecule's flexibility, the less likely it is to be active when administered orally (VEBER *et al*., 2002).

Table 2 represents the values of molecular descriptors obtained after use of the Molinspiration Cheminformatics® online database.

Table 2: Assessment of the Oral Bioavailability Profile.

Analog	**Caffeic Acid**	**Ferulic Acid**	**p-Cumaric acid**	**Synapic Acid**
miLogP	0.94	1.25	1.43	1.26
PM	180.16	194.19	164.16	224.21
SDLH	3.	2.	2.	2.
SALH	4.	4.	3.	5
TPSA	77.75	66.76	57.53	76
Violations*	0	0	0	0
NLR*	2.	3.	2.	4.
VM***	154.5	172.03	146.48	197.57

Source: Molinspiration Cheminformatics®
* Violations: Violations of Lipinski's Rule; ** NLR: Number of Rotatable Links; *** VM: Volume Molecular.

Table 2 indicates extremely promising results for all cinnamic acid derivatives, as the four derivatives studied did not violate Lipinski's Rule and have good prediction of bioavailability profile when administered orally.

3.3 PHARMACOKINETIC STUDY *IN* HUMAN *SILICO* (ADME *IN SILICO*)

In this stage of the work, the ADME study *in* human *silico was conducted* to predict the following molecular parameters for the four cinnamic acid analogs: Human Intestinal Absorption (HIA), Permeability by the Blood Brain Barrier (BBB), Permeability by Caco-2 epithelial cells, Inhibition of P-glycoprotein and Cell distribution of compounds in the human organism.

It is important to note that a certain drug administered orally is transported through the bloodstream and will then be distributed into the tissues of the human body to the receiving targets. To do so, their molecules need to permeate through various barriers that delimit the external medium of the internal medium. Therefore, for an orally administered drug to exert action on the central nervous system (CNS), barriers including intestinal wall tissues, intestinal capillary walls, and the blood-brain barrier should be permeated.

It is worth mentioning that the membrane systems are amphipathic (amphiphilic) in nature, that is, they form a double layer of phospholipids with a hydrophilic surface (external medium) and with the hydrophobic interior medium in which membrane proteins are embedded. Membrane phospholipids are amphiphilic as to solubility because the fatty acid chains are hydrophobic and the outer polar head is hydrophilic. Therefore, the hydrophobic interior of the membranes facilitates the permeability of the nonpolar drugs and makes the passage of polar molecules, in particular the ionized ones, difficult. The ability to cross the double lipid layer is a prerequisite for the absorption of the analogous compounds under study, as they should cross the blood-tissue barrier (capillary endothelium), the plasma membranes (plasmalema) into the cells and permeability through the membranes of the cellular organelles.

Absolute bioavailability of a drug is defined as the portion of the dose administered orally that reaches the systemic circulation intact. Absolute bioavailability depends on several factors, among which are: 1) Drug delivery system of presentation form (pharmacotechnical form); 2) Drug permeability dissolved by the epithelial membrane at the site of absorption performed in the gastrointestinal tract; and 3) Loss performed by presystemic metabolism (GOLAN *et al*., 2017).

In pharmacokinetic terms, human intestinal absorption (HIA) represents the sum of the absorption rate and absolute bioavailability of the drug that reaches the systemic circulation, because when administered orally the drug concentration will always be less than 100% due to incomplete extent in the absorption process and elimination regarding the first-pass effect (hepatic biotransformation). Thus, the %HIA represents the percentage of dose of the active ingredient that was administered orally and that reaches the hepatic portal system (HOU and WANG, 2008).

In the Central Nervous System (brain and spinal cord), endothelial cells have no pores and have little transcytotic activity. For a given analog to cross the blood-brain barrier, it will need to pass through endothelial cells and permeate through the luminal and basal membranes. This membrane permeability requires specific physicochemical properties with respect to the compound and depends on the transport mechanism of the compound. The permeability of chemical compounds through the blood-brain barrier (BBB) consists of passage through endothelial cells that have tight and compacted junctions, which considerably restricts the permeability of the compounds, ensuring that the drug candidate does not cross the BBB, otherwise it would considerably increase the probability of presenting side effects (adverse effects) (THOMAS, 2003). In the capillary endothelium of the cerebral vessels the presence of P-glycoprotein is fundamental, since it acts as a defense mechanism with the capacity to pump back into the blood xenobiotic chemicals that could possibly cross the blood-brain barrier (THOMAS, 2003), (PATRICK, 2010).

Currently, the FDA (*Food and Drug Administration*) has recommended the study *in silico* using Caco-2 epithelial cells, as it is an estimative parameter of intestinal permeability due to morphophysiological similarity with human enterocytes. Depending on the complexity of the absorption of compounds in the gastrointestinal tract, the prediction of permeability by Caco-2 epithelial cells (from colon adenocarcinoma) was also evaluated.

Table 3 shows the evaluation of the pharmacokinetic profile *in* human *silico* (ADME *in silico) of the molecular descriptors: Human Intestinal Absorption (HIA), Permeability by the Blood Brain Barrier (BBB), Permeability by Caco-2 epithelial cells, Inhibition of P-glycoprotein and Cell distribution of derivatives in the body.* Data are presented qualitatively [(Q - P: positive or N: negative)] and quantitatively (P = probability) for the four cinnamic acid analogs.

Table 3: Pharmacokinetic profile assessment *in silico* human

Analogs	**BBB**		**HIA**		**Distribution**		**P-glycoprotein inhibitor**		**Caco-2**	
	Q	**P**	**Q**	**P**	***Org.**	**P**	**Q**	**P**	**Q**	**P**
Caffeic Acid	N	65.0%	P	97.4%	**Mit.	80.3%	N	99.1%	P	50.0%
Ferulic Acid	N	65.0%	P	98.4%	**Mit.	83.3%	N	98.7%	P	58.0%
p-Cumaric acid	N	77.5%	P	99.6%	**Mit.	82.3%	N	98.9%	P	85.3%
Synapic Acid	N	55.0%	P	98.0%	**Mit.	81.2%	N	96.3%	P	52.9%

Source: admetSAR®.
* Org.: Organelle; ** Mit: Mitochondria.

The evaluation indicated that all cinnamic acid analogs were positive for HIA, with high rates of intestinal absorption, with caffeic acid showing the lowest rate of intestinal absorption (97.39%) and p-cumaric acid the highest rate of intestinal absorption equivalent to 99.59%, i.e., as all analogs present HIA much higher than 70%, therefore they are classified as compounds with high rate of intestinal absorption (MATOS, 2017). The increasing order of human intestinal absorption (HIA) for cinnamic acid derivatives is: caffeic acid < synaptic acid < ferulic acid < p-cumaric acid.

HIA (*Human intestinal absorption*) is an important parameter of the intestinal absorption of the analog. The HIA parameter evaluation takes into consideration the following values regarding intestinal absorption: 1) Between 0 and 20%: low absorption; 2) Between 20% and 70%: moderate absorption; 3) Greater than 70%: high absorption rate (YAKAIAH *et al*., 2015). Therefore, as can be analyzed in table 3, all analogs have high levels of intestinal absorption, since all present HIA higher than 97%.

Subsequently, the prediction of the parameter correlated with the Permeation by the Blood Brain Barrier (BBB) was evaluated. This descriptor is associated with the permeability of the compounds by endothelial cells that considerably restrict the passage of compounds transported by the bloodstream to the CNS (SHARMA *et al.*, 2016), (DOLABELA *et al*., 2018) and (KANAZAWA, 2018). When analyzing table 3, it is noted that all cinnamic acid analogs were negative for permeability by BBB, i.e., none of the derivatives cross the blood brain barrier, which considerably reduces the probability of causing side effects by xenobiotics. With regard to the cellular distribution of the derivatives of cinnamic acid, all the analogs had as their target the mitochondria, the organelle responsible for cellular respiration.

Liver metabolism or hepatic biotransformation is a process that promotes alteration in the chemical structure of the compound resulting from biochemical reactions that generate products called metabolites. Liver biotransformation, along with excretion, are pharmacokinetic components responsible for drug elimination.

Hydrophilic drugs (high polarity) are easily excreted in urine due to high plasma solubility and poor reabsorption in renal tubules. If the derivative is ionized, urinary excretion will also be increased, with greater secretion in the renal tubules, as the compound will not be bound to plasma proteins, causing an effective increase in glomerular filtration rate. Many compounds have high hydrophobicity (lipophilicity), so they need to be metabolized to hydrophilic compounds before they can be excreted via urine.

The terms lipophilic/hydrophobic and lipophobic/hydrophilic refer respectively to the solubility of the compounds in polar and polar media. Importantly, blood plasma, interstitial fluid, and cellular cytoplasm are highly polarized. When hydrophilic drugs reach the bloodstream, they slowly pass through the lipid membrane of the hepatocyte in the liver without altering its chemical structure and have no access to liver enzymes.

The liver is the major site of drug biotransformation, with the three major components in the metabolism process being: the reagents (drug or xenobiotic), the product (metabolite), and the catalyst of the biochemical reaction (liver enzymes). Hepatocytes have an enzyme apparatus responsible for hepatic metabolism, located on the membranes of the smooth endoplasmic reticulum (REL) and rough endoplasmic reticulum (RER).

REL enzymes, microsomal enzymes, or mixed-function oxidases are called cytochrome P450 complex enzymes. These enzymes are very important in the biotransformation of drugs and catalyze many hydroxylation and oxidative hydrolysis reactions of type -N-C- or -0-C- chemical bonds by metabolizing a variety of lipophilic compounds. The cytochrome P450 complex enzyme superfamily is subdivided into families (eg. CYP3), subfamilies (e.g. CYP3A) and finally isoenzymes (e.g. CYP3A4) according to the primary amino acid chain structure of the enzyme.

The last step of the ADME *in silico study* was the assessment of the *in silico* pharmacokinetic profile of analogs to predict inhibition and interaction with hepatic cytochrome P450 complex enzymes (CYP450) in hepatic metabolism. Table 4 qualitatively (I = yes/no) and quantitatively (P = probability) represents the evaluation of

the *in silico* inhibitory pharmacokinetic profile of hepatic cytochrome P450 isoenzymes by cinnamic acid analogs.

Table 4: Assessment of the pharmacokinetic profile *in* human silico (ADME *in silico*[1]*) inhibitory of cytochrome P450 complex isoenzymes (CYP450).*

Analogs	**CYP450 1A2**		**CYP450 2C9**		**CYP450 2D6**		**CYP450 2C19**		**CYP450 3A4**	
	I.	**P**	**I.**	**P**	**I.**	**P**	**I.**	**P**	**I.**	**P**
Caffeic Acid	**not**	90.4%	**not**	61.1%	**not**	86.8%	**not**	93.6%	**not**	88.7%
Ferulic Acid	**not**	75.1%	**not**	57.9%	**not**	85.0%	**not**	62.7%	**not**	92.4%
p-Cumaric acid	**not**	94.5%	**not**	93.6%	**not**	97.6%	**not**	91.2%	**not**	86.9%
Synapic Acid	**not**	84.4%	**not**	83.8%	**not**	92.9%	**not**	71.8%	**not**	87.5%

Source: admetSAR®.

The analysis in table 4 provides relevant information regarding inhibitory prediction of cytochrome P450 complex enzymes. It is noted that none of the four cinnamic acid analogs have inhibitory capacity of the five isoenzymes of the CYP450 families, that is, the analogs do not affect hepatic metabolism, facilitating the excretion of xenobiotic and/or lipophilic compounds from the organism (GALLI and FEIJOO, 2002). Compounds that have inhibitory capacity for some hepatic isoenzyme may result in a number of biochemical processes and adverse effects, which will impact the hepatic metabolism of other drugs, formation of toxic metabolites, and changes in the genetic formation of several enzymes. DEVLIN, 2002.

3.4 TOXICOLOGICAL STUDY *IN* HUMAN SILICO

The human *in silico* toxicology study for cinnamic acid analogs was conducted to predict AMES toxicity (T: toxic; NT: non-toxic), carcinogenicity (C: carcinogenic; NC: non-carcinogenic) and acute oral toxicity of derivatives in categories (I, II, III and IV).

The AMES test is a bacterial test using the strain *Salmonella typhimurium*, (TA100 and TA1535) to assess the mutagenicity of the compound (MIRANDA *et al.*; 2021). From this test, it is possible to detect mutations in the genetic material involved in the synthesis of the amino acid histidine (KAUFFMANN *et al.*, 2020).

For the assessment of Acute Oral Toxicity, derivatives were distributed into 4 categories. Category I (high toxicity) represents compounds with LD50 (50% of the Lethality Dose in: mg derivative/kg body weight) less than or equal to 50 mg/kg. Category II (moderate toxicity) represents compounds with LD50 with values greater than 50 mg/kg and less than 500 mg/kg, while Category III (low toxicity) includes compounds with LD50 values greater than 500 mg/kg and less than 5000 mg/kg, and Category IV (non-toxic) includes compounds with DL50 values greater than 5000 mg/kg. Table 05 provides qualitative (Q = NT/NC and C) and quantitative (P) information regarding the assessment of the toxicological profile *in* human *silico* of cinnamic acid analogs.

The analysis in table 5 shows that all cinnamic acid analogs are non-toxic for human toxicity assessment based on the AMES test, non-carcinogenic and for acute oral toxicity category III and IV (non-toxic).

Table 5: Evaluation of the Toxicological Profile *in* Human silico.

Analog	**AMES** ***Q **P**		**Carcinogenic** ***Q **P**		**Acute Oral Toxicity** *****C **P**	
Caffeic Acid	**NT**	91.0%	**CN**	78.2%	**IV.**	55.9%
Ferulic Acid	**NT**	99.0%	**CN**	71.9%	**IV.**	62.7%
p-Cumaric acid	**NT**	94.0%	**CN**	61.1%	**III.**	49.0%
Synapic Acid	**NT**	82.0%	**CN**	71.8%	**III.**	45.0%

Source: admetSAR®.
* Q: Qualitative; **P: Probability *** C: Category.

4 CONCLUSION

The present study made it possible to predict through *in silico* methodologies *that* the four cinnamic acid analogs (caffeic acid, ferulic acid, p-cumaric acid and synaptic acid) are considered promising drugs for the development of medicines, as they presented good oral availability, favorable pharmacokinetic parameters and toxicological effects without side effects and low toxicity. Previously reported *in vitro* and *in vivo* studies reported from the literature as anti-inflammatory, antioxidant, anticancer and antimicrobial activities (in addition to other biological activities) reveal the impact of this research for the four cinnamic acid-derived analogs. Molecular Modeling performed for the cinnamic acid analogs revealed that the hybrid method used (Energy Minimization:

PM3 + Geometric Optimization: DFT) considerably reduced the steric energy of all derivatives, resulting in chemical structures of lower energy (local minimum: greater stability). Virtual *Screening* for oral bioavailability revealed that all analogs did not violate Lipinski's rule. The ADME study *in* human *silico* for prediction of pharmacokinetic parameters indicated that all derivatives exhibit high intestinal absorption rate (HIA), do not cross the blood brain barrier (BBB), are permeable by Caco-2 cells, and are not inhibitors of P-glycoprotein. As for the ADME profile *in silico* human with regard to the inhibitory prediction of hepatic cytochrome P450 complex enzymes (CYP450), it is noted that the four analogs do not act as inhibitors of hepatic isoenzymes tactics. The Toxicological study *in* human *silico* also proved relevant, as all analogs are non-toxic for AMES testing, non-carcinogenic and acute oral toxicity fall into categories III and IV (non-toxic). Therefore, it can be stated that cinnamic acid analogs are natural compounds qualified for further studies of *in silico* pharmacodynamic prediction, study of the quantitative relationship between chemical structure-biological activity (QSAR) and *Structure Based Drug Design* (SBDD)-type studies *such as molecular docking and molecular dynamics to elucidate ligand-receptor intermolecular interactions.*

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